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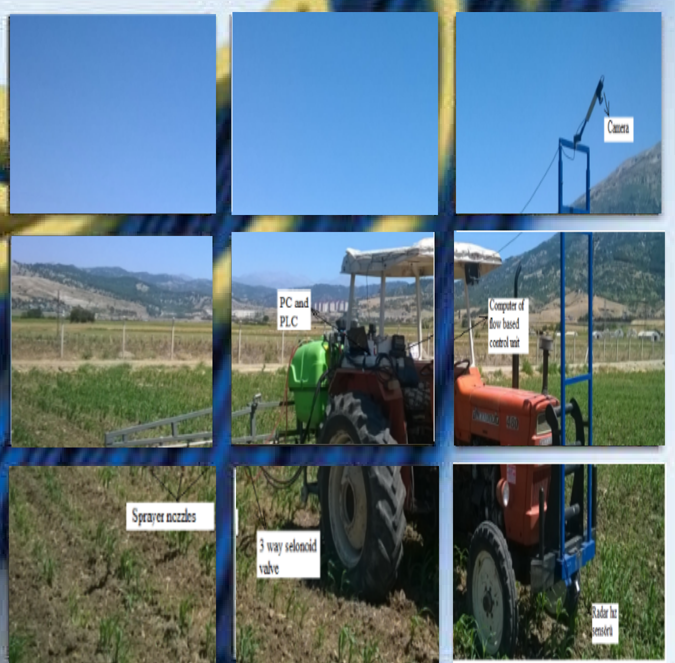

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Typhlodromus (*Anthoseius*) *caudiglans* (Schuster) (Acari: Phytoseiidae), The New Record for the Predatory Mite Fauna of Turkey in Erzurum

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ABSTRACT
Typhlodromus (*Anthoseius*) *caudiglans* (Schuster) (Acari: Phytoseiidae) was collected from *Hippophae salicifolia* L. (Elaeagnaceae) leaves in Erzurum during the years 2015-2016. *T.* (*A.*) *caudiglans* is a predatory mite lives on ornamental plants. The samples were extracted by Berlese funnel and cleared in Lacto-phenol solution after that mounted in Hoyer solution. The samples were deposited in the mite collection at Ankara University and Atatürk University Plant Protection Department of Turkey.

This is the first record of *T.* (*A.*) *caudiglans* for Phytoseiidae fauna of Turkey. Re-description and illustration of the new record is given.

Keywords: Elaeagnaceae; Mesostigmata; Turkey; Taxonomy; *Typhlodromus* (*Anthoseius*) *caudiglans*

1. Introduction

Phytoseiidae (Acari) fauna is pretty well known in Turkey, comparison to the other European countries. 103 Phytoseiid species were designated which were presented by *Amblyseius* Berlese (12), *Aristadromips* Chant & McMurtry (1), *Chelaseius* Muma & Denmark (1), *Eharius* Tuttle & Muma (3), *Euseius* Wainstein (4), *Galendromus* Muma (1), *Graminaseius* Chant & McMurtry (1), *Iphiseius* Berlese (1), *Kampimodromus* Nesbitt (4), *Typhlodromus* (*Typhlodromus*) Scheuten (13), T*yphlodromus* (*Anthoseius*) De Leon (14), *Metaseiulus (Metaseiulus*) Muma (1), *Neoseiulus* Hughes (18), *Neoseiulella* Muma (3), *Paragigagnathus* Amitai & Grinberg (1), *Paraseiulus* Muma (4), *Phytoseiulus* Evans (2), *Phytoseius* Ribaga (6), *Transeius* Chant & McMurtry (4). *Proprioseiopsis* Muma (3), *Typhloseiella* Muma (2), *Typhloseiulus* Chant & McMurtry (3). France (90), Germany (78) while Belgium has (18) species. (Demite et al 2015; Döker et al 2016). Some phytoseiid species were reported in Erzincan and Erzurum; *T*. (A.) *kazachstanicus* Wainstein (Ecevit 1981); *Euseius finlandicus* (Oudamans), *Kampimodromus aberrans* Oudemans, *Paraeiulus soleiger* (Ribaga), *Parasiulus talbii* (Athias-Henriot), *Phytoseius echinus* Wainstein and Arutunjan, *Neoseiulella tiliarum* (Oudemans), *Typhlodromus (Anthoseius) rhenanus* (Oudemans) (Alaoğlu 1996 a, b), *Neoseiulus zwölferi* (Dosse) and *Proprioseiopsis okanagensis* (Chant) were reported from Erzurum (Çobanoğlu 1989a, b; Kasap & Çobanoğlu 2007; 2009; Faraji et al 2011).

Survey studies can provide detection of a predatory mite; *Typhlodromus* (*Anthoseius*) *caudiglans* (Schuster) (Acari: Phytoseiidae) species on ornamental plants.

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The aim of this study was re-description of *T.* (*A.*) *caudiglans* determined from ornamental plants in Erzurum 2015-2016.

2. Material and Methods

The samples were collected from ornamental plants in Erzurum which are located Eastern part of Turkey, during 2015 and 2016 (Figure 1).

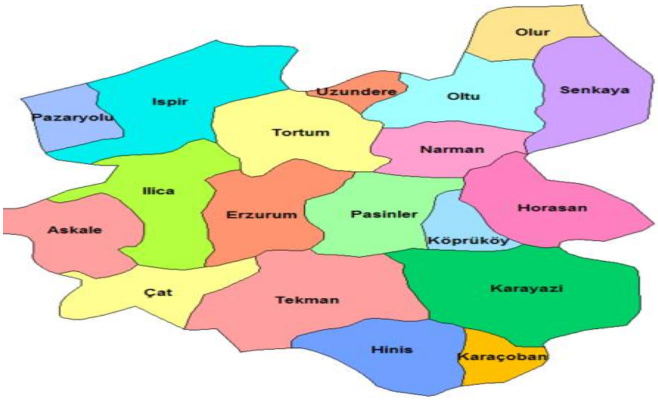


Figure 1- Sampling localities: Erzurum (Eastern Part of Turkey) (★)

The mite samples were extracted by Berlese funnel. They were cleared in Lacto-phenol solution and mounted in Hoyer's medium, afterwards they dried for 2-3 weeks at 50 °C (Henderson 2001).

The identification and drawing of the mites were done by using Leica DM 2500 microscopes.

The idiosoma was measured from the base of the gnathosoma to end of opisthosoma. The length of setae were measured from their bases to their apex. The setae measurements are given in micrometres and average followed by the range in parentheses. The plant samples collections were made by K. Akçakoyunluoğlu (Atatürk University).

All the identification of the samples were made by Sultan Çobanoğlu, according to; Kolodochka (1978), Chant (1957), Chant et al (1974; 1978). Measurements and World distribution, of the species, is provided.

3. Results and Discussion

Phytoseiidae
Typhlodrominae
Typhlodromini

Typhlodromus (*Anthoseius*) De Leon 1959
Typhlodromus (*Anthoseius*) *caudiglans* (Schuster 1959)
Anthoseius timidus Wainstein et Arutunjan (1968).
Typhlodromus (*Anthoseius*) *nodosus* (De Leon 1962) - Lehman (1982)
Typhlodromus (Anthoseius) caudiglans (Schuster) Chant et al (1978)
Typhlodromus (Typhlodromus) caudiglans (Schuster) - Chant et al (1974)

Female (n= 5)
(Figures 2-6, 7-15)
Dorsal idiosoma (Figures 2, 7, 10)
Idiosomal setal pattern: 12A: 8A (included r3 and R1).

Dorsal shield: 343 (330-350) long, 237 (200-290), width at j6 level, elongate, oval and ornamented and imbricated all the dorsal shield, with distinct lateral notches. with 20 pairs of dorsal setae. The dorsal setae mostly smooth, except Z_4 and Z_5, slightly serrated, and Z5 knobbed apically lengths j_1 18 (15-20), j_3 17 (15-18), j_4 14 (13-15), j_5

14 (13-15), j_6 16 (15-18), J_2 19 (18-20), J_5 10 (8-13), z_2 17 (15-20), z_3 21 (18-25), z_4 18 (18-20), z_5 16 (15-18), Z_4 32 (30-35), Z_5 53 (50-55), s_4 24 (23-28), s_6 25 (23-28), S_2 29 (28-33), S_4 34 (33-35), S_5 29 (25-33), setae r3 20 (15-23), R_1 20 (18-23), placed on lateral integument. Five pairs of large visible soleostome (gd_2, gd_4, gd_6, gd_8 and gd_9), and 4 pairs of relatively large and conspicuous poroids exist on the dorsal shield. Z_5 is the longest seta among the other dorsal setae with a very weak clava.

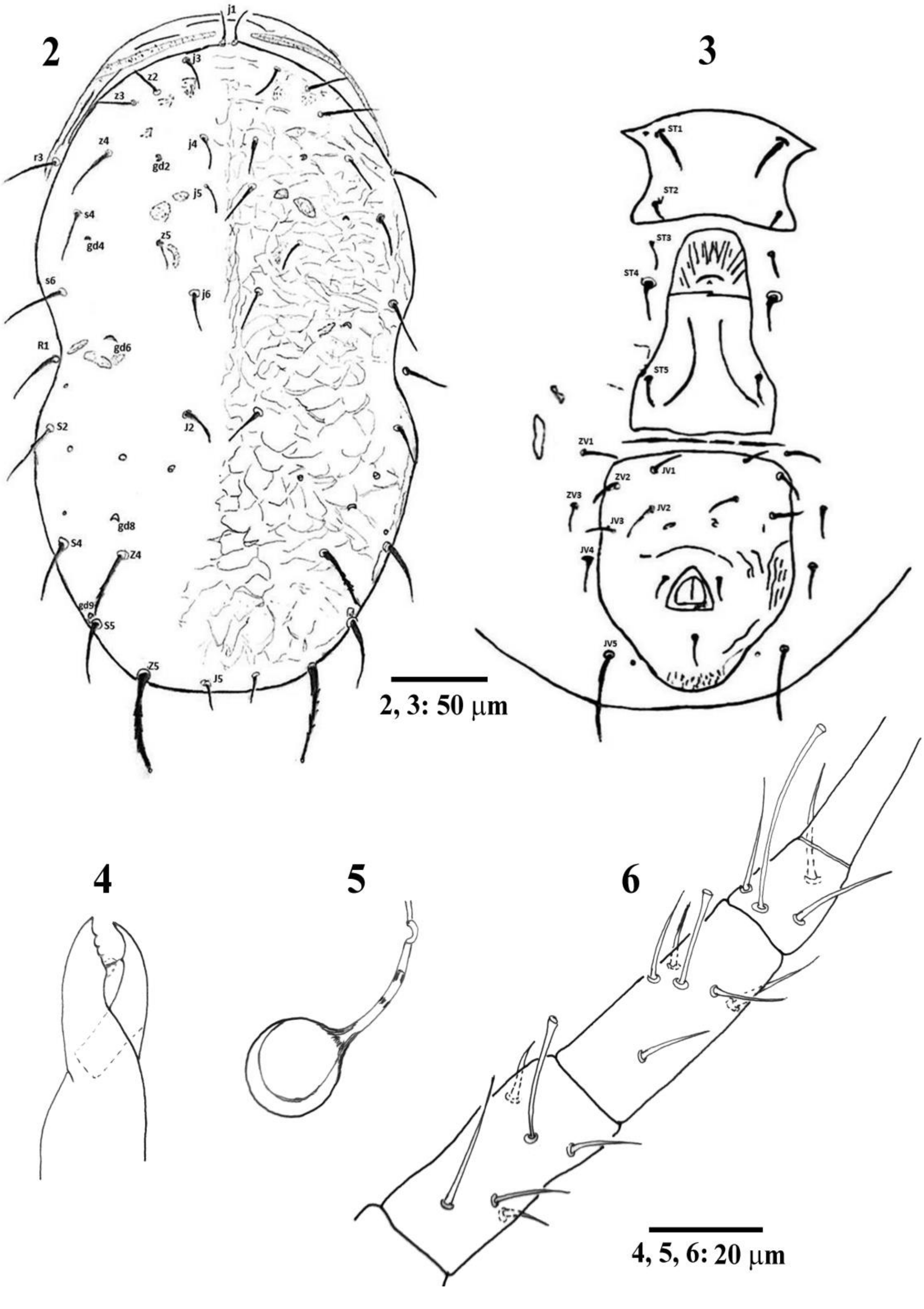
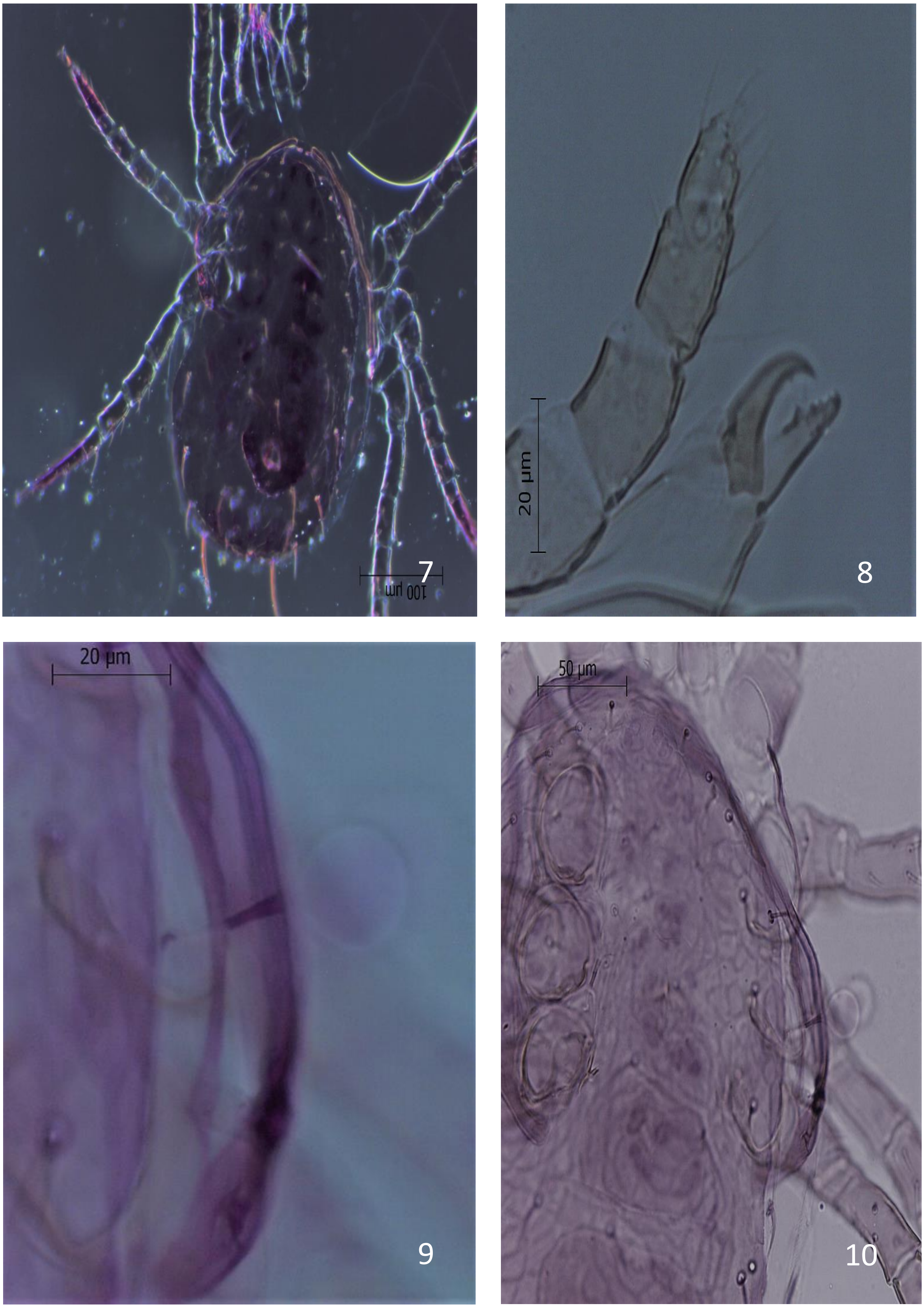
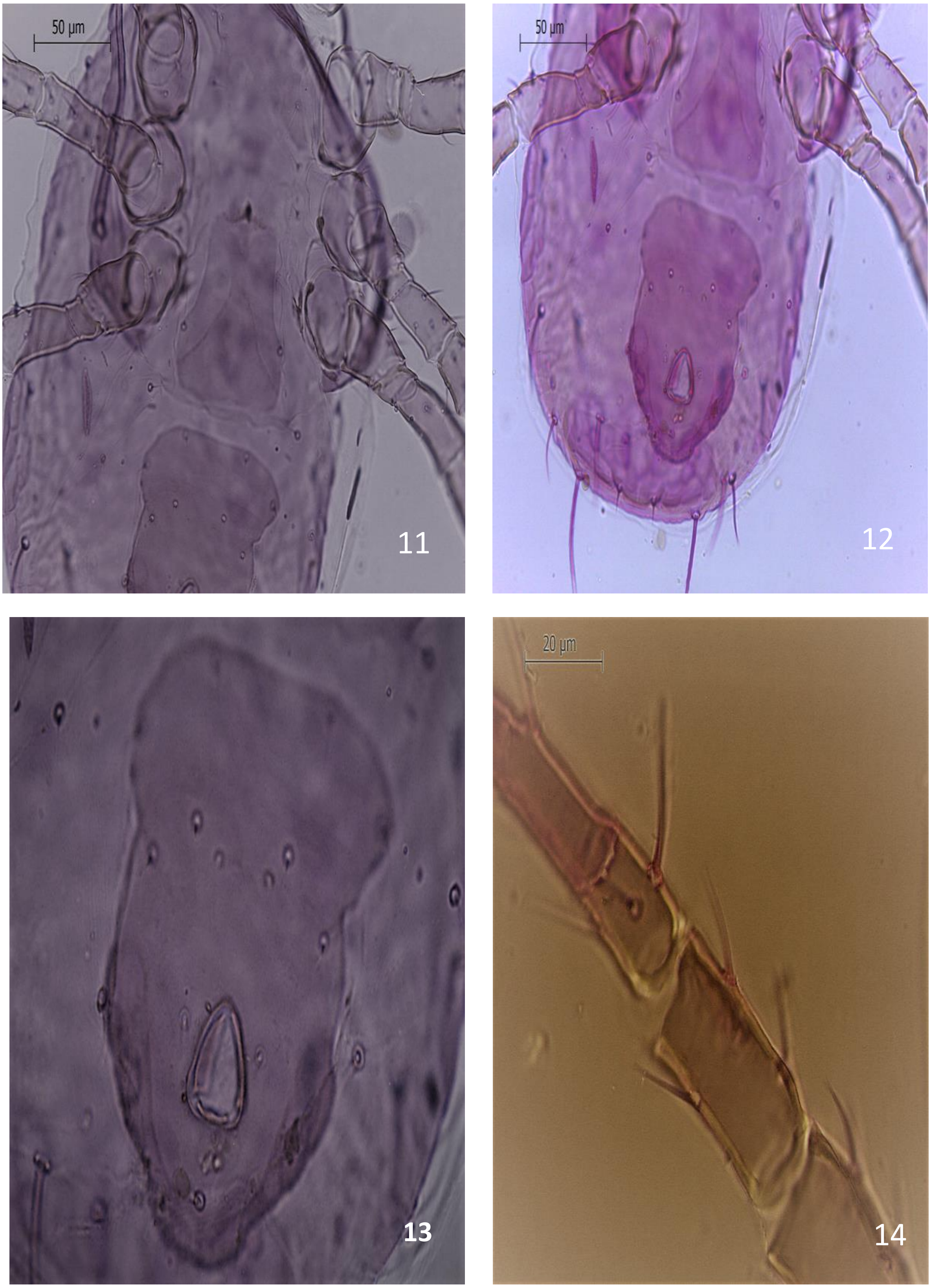


Figure 2-6. *Typhlodromus* (*Anthoseius*) *caudiglans* (Schuster), female 2. Dorsal view 3. Ventral view 4.Chelicera 5. Spermathecae 6. Genu, tibia and basitarsus IV



Figures 7-10- *Typhlodromus* (*Anthoseius*) *caudiglans* (Schuster), female; 7, Dorsal view; 8, Chelicera; 9, Peritrem; 10, Dorsal reticulation, peritrem



Figures 11-14- *Typhlodromus* (*Anthoseius*) *caudiglans* (Schuster), female; 11, Ventral view; 12, Ventral opisthosoma; 13, Ventrianal plate; 14, Genu, tibia and basitarsus IV

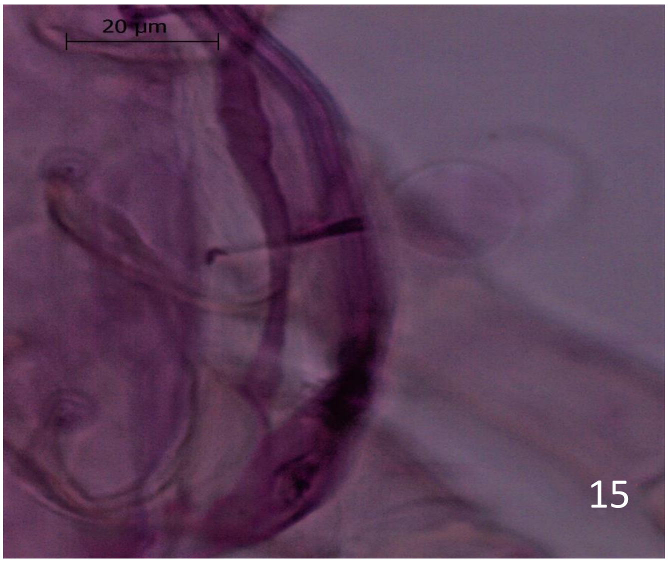


Figure 15- *Typhlodromus* (*Anthoseius*) *caudiglans* (Schuster), female; 15, Spermatheca

Peritreme - Extends to the insertions of setae j_1 (Figure 2, 9, 10).

Ventral idiosoma - (Figure 3, 11-13) - Sternal plate smooth with, 63 (55-68) long and 63 (55-68) width at level of setae ST_2, with two pair pores (iv_1 and iv_2 at tip of the shield) and 2 pairs setae, ST_1 19 (18-20) and ST_2 19 (15-23) and ST_3 15 (13-18) on interscutal membrane, ST_4 15 (13-18) on small platles with a pair of pores, genital plate smooth at middle, 64 (58-73) wide at widest point, ST_5 20 (15-25); with 2 pairs of metapodal plates, the primary 28 (25-30) long-ovoid and accessory 9 (8-13) long; ventrianal plate sub pentagonal shaped and well sclerotized with some striae between JV_2 and paranals, with clearly defined waist in some specimen while there is not very clear in some others, and round shape preanal pores set close together each other; length 113, and 93 (90-95) width at setae ZV_2; and level preranals 83 (78-88) width; with 4 pairs of preanal setae JV_1 8 (8-10), JV_2 8 (5-10), JV_3 7 (5-8), ZV_2 12 (8-18); with a pair of muscle marks lateral of anal opening; distance between these pores 22 (20-23), 4 pairs of setae surrounding ventrianal shield on integument, JV_4 15 (13-15), JV_5 38 (35-43), ZV_1 13 (13-15), ZV_3 15 (10-18); with five pairs of small pores and poroids surrounding integument. There is a sclerotized line and folds between genital and ventrianal shield. Some species show substantial variation in ventrianal shield, regularly intended, subrectangular with a pair of pore (Figure 3, 11-13).

Spermatheca **-** Calyx long and tube-shaped, tubular and well sclerotised 11 (8-15) length, width 14 (13-15) (Figure 5, 15).

Chelicera-Fixed digit 26 (25-28) length with 3 small teeth, with pilus dentilis; movable digit 24 (23-28) length have a tooth (Figure 4, 8).

Legs-Leg IV (Figure 6, 14) basitarsus with small knobbed macrosetae, clavate at the tip, *St IV* 30 (28-30); the measurement from the base of macrosetae to slit-like organ 42 (40-43), usually genu, tibia and basitarsus IV with one macrosetae; the chaetotactic formulae of genua and tibiae, I–II–III–IV with 10-7-7-6 and 10-7-7-6 setae respectively.

Material examined **-** Aşkale-Çayköy, 11.VII.2015 (5 ♀♀); 06.VI.2015, (6 ♀♀) (*Hippophae salicifolia* L. (*Elaeagnaceae*), (39°56’03.1” N, 40° 43’ 32. 8” E, 1662 m).

Distribution: Austria, Azerbaijan, Canada China, Czechia, England, Iran, Latvia, Lithuania, Moldova, New Zealand, Norway, Russian Federation, Slovakia, Ukraine and USA (Chant et al 1978; Demite et al 2015; Moraes et al 2004); and Turkey (new record).

Host Plants: alder, apple, ash, birch, blubbery, cherry, cedar trees, elm, hawthorn, hazel, *Malus* sp., mulberry, maple, oak, peach, plum, poplar berry, pine poplar, *Prunus* sp., *Tamarix* sp., willow (Chant et al 1974; Kolodochka 1974; 1978).

Remarks: *T. (A.) caudiglans*, is a new report for the predatory mite fauna of Turkey. This species is very close to *Typhlodromus* (*Anthoseius) krimbasi* Papdoulus & Emmanuel, with a bulbous tip of setae Z5 and similar shape of spermatheca. *T.* (*A.*) *caudiglans* has been separated from latter by the length of dorsal setae and most of them smooth except Z_4 and Z*5*, one tooth on movable digit of chelicera; ventrianal shield with rounded small pores. *T.* (*A.*) *krimbasi*, with 3 teeth on a movable digit and crescent shape solenostomes on the ventrianal shield. *T. (A.) caudiglans,* is separated from *T.* (*A.*) *rapidus* Wainstein & Arutunjan, by the movable digit of chelicera with three teeth in latter. The measurements of dorsal setae and the ventral features of Turkish specimens have concurred with Canadian specimen (Chant et al 1974; 1978). In our specimen, fixed digit has 3 teeth and pilus dentilis. Kolodochka (1978) mentioned that fixed digit with four teeth and *Pilus dentilis* for Ukraine specimen (Table 1).

Table 1- Some taxonomical charateristics and setae lengths *Typhlodromus* (*Anthoseius*) *caudiglans* (depends on different countries) and *Typhlodromus* (*Anthoseius*) *krimbasi*

Characteristics	*Typhlodromus (A.) caudiglans (Turkey)*	*Typhlodromus (A.) caudiglans (Ukraine) (Kolodochka 1978)*	*Typhlodromus (A.) caudiglans (Canada) (Chant et al 1974)*	*Typhlodromus (A.) krimbasi (Greece) (Papadoulis & Emmanouel 1997)*
j_3	17	14	24	26
J_2	19	19	-	36
z_4	18	19	25	37
Z_4	32	28	28	49
Z_5	53	45	48	62
s_4	24	22	25	39
s_6	25	24	31	43
S_2	29	25	30	47
S_4	34	22	32	48
S_5	29	22	28	42
Number of solenostome on dorsal shield	5	5	1	5
Teeth/Fixed digit	3	4	2-3	4
Teeth/movable digit	1	1	1	3
Macroseta on tarsi ıv.	30	25		29
Ventrianal pores	A pair rounded pore	A pair rounded pore	A pair of distinct pore	A pair crescent shape pore

As a conclusion, *T.* (*A.*) *caudiglans* was identified as a new record for the mite fauna of Turkey. The biodiversity of Phytoseidae family is very rich in Turkey, especially it is very important to further detailed studies on ornamental plants in Erzurum. It is a great advance to take into account Phytoseiidae species for controlling of the plant-parasitic mites especially depending on with native predatory mite species for protecting the environment and controlling the pesticide resistance problem.

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Assessment of Irrigation Schemes with Performance Indicators in Southeastern Irrigation District of Turkey

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ABSTRACT
Water resources are among the most important natural riches of the countries, so this resource must be used correctly and sustainable, especially in the agriculture sector. The Southeastern District of Turkey, has abundant water resources but is known as a region where the problems arising from the excessive use of water. In this study, 5 irrigation schemes (Akçakale, Şanlıurfa, Yaylak Plain, Upper Harran, Bozova) in the Southeastern District of Turkey were chosen as a material to investigate the performance. It is aimed to assess the irrigation schemes in the district with performance indicators used widely by researchers. As an assessment method, ANOVA was used to determine differences of performance indicators among irrigation schemes, multiple regression and correlation were used to explain statistical relation among performance indicators. As a result, irrigation water supplied to users per unit irrigated area ($W_{irrigated}$) can be explained with irrigation ratio (I_{ratio}), irrigation water supplied to users per unit command area ($W_{command}$), relative water supply (R_{ws}) and output per unit irrigation water supplied to users (O_{water}) (R^2= 0.98). In the region, the average R_{ws} was found 2.38 although irrigation methods used by farmers were generally sprinkler. The study also concluded that serious operation, maintenance and management problems exist in the irrigation schemes of the district.

Keywords: ANOVA; Irrigation schemes; Performance assessment; Multiple regression analysis

1. Introduction

Approximately 70% of the freshwater in Turkey is consumed in agriculture. Irrigation associations, irrigation cooperatives, village legal entity are responsible for the management of water used in agricultural activities (DSI 2017). The effective use of irrigation water has a vital role in reducing the effects of water scarcity due to global warming (Vörösmarty et al 2000; Flörke et al 2018). In this context, monitoring and evaluation of irrigation schemes are of essential (Degirmenci 2001) in assessing the performance of irrigation schemes. The researchers developed a number of performance indicators for evaluating irrigation schemes (Molden et al 1998) and these indicators were improved with the following studies (Burt 2001; Malano et al 2004) including methodology for modernizing irrigation management (Renault et al 2007). These performance indicators provide an overview of the performance in irrigation schemes. Evaluation of irrigation schemes are also necessary in Turkey, the use of performance indicators used by researchers in many studies aimed to identify strengths and weaknesses of the irrigation schemes (Tanriverdi et al 2011; Degirmenci et al 2017; Elicabuk & Topak 2017; Arslan & Degirmenci 2017; Arslan & Degirmenci 2018; Kiziloglu et al 2018). The evaluation of irrigation schemes with the performance indicators are also widely used in other countries (Rodriguez-Diaz et al 2004; Denis et al 2017; Alcon et al 2017; Zema et al 2018; Muema et al 2018).

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To increase the expected benefits of irrigation schemes, it is necessary to conduct various analyses at regular intervals. Researchers used and developed different analysis methods to evaluate and improve the performance of irrigation schemes. Data envelopment analysis techniques were used to complete deficiency in the interpretation of performance indicators (Rodriguez-Diaz et al 2004). They calculated performance indicators and analyze with multivariate data analysis in some irrigation schemes (Rodriguez-Diaz et al 2008) created the new analysis called quality index including principle component analysis and cluster analysis allows ranking irrigation schemes based on their success. Similar studies carried out by Corcoles et al (2010); Corcoles et al (2012) in Castilla-La Mancha (Spain), Zema et al (2015); Zema et al (2018) in Calabria (Italy) and Kartal (2018) in all irrigation schemes in Turkey, Alcon et al (2017) in Segura River Basin (Spain). All studies indicated the importance of evaluation irrigation schemes in terms of agricultural water management in the world. In parallel studies in Turkey, Cakmak et al (2004) conducted a study in the southeastern part of the country, Tanriverdi et al (2011) assessed the effects of management types of the irrigation schemes, Uysal & Atis (2010) evaluated Kestel Water User Association in Bursa. Studies done by Kiziloglu et al (2018), Arslan & Degirmenci (2018), Kalender & Topak (2017) have been another example of evaluation of irrigation schemes by different performance indicators in Turkey.

The Southeastern District of Turkey is of great importance in terms of irrigation development. This region is located in the Southeastern Anatolian Project (GAP) area wherein the most significant economic and social development project of Turkey erected. Overuse of irrigation water in this region is a major problem due to the lack of infrastructure facilities and management skills (Kartal 2018). In his study, the irrigation schemes in the district were ranked with the overall performance score not separately. The irrigation performance of the region is far from the desired level of success; thus there is need to investigate the district closely Assessment of irrigation schemes by the performance indicators in the district may serve the achievement of sustainable agriculture goals and facilitate the improvement of irrigation performance for decision makers and irrigation managers.

The main aim of this study is to investigate the district including 5 irrigation schemes (Akçakale, Şanlıurfa, Yaylak Plain, Upper Harran, Bozova) located in the Southeastern District of Turkey. The study also focuses on explaining the strengths and weaknesses of them. Eight selected performance indicators among many others calculated for 10 years period and statistical relationships among performance indicators were investigated with ANOVA, correlation and multiple regression analysis in the study.

2. Material and Methods

2.1. Description of study area

The 15th (Southeastern) District located in GAP area among all 26 Regional Directorates of State Hydraulic Works (DSI) Districts was chosen as the study area. The main characteristics of the irrigation schemes in the study area were given in Table 1. GAP is the largest regional development project in Turkey. GAP is an integrated and multi-purpose project aiming to provide social and economic development of the region by mainly irrigation and energy investments including Euphrates and Tigris Basins in 15th District of DSI (DSI 2017). This project, which is one of the most significant investments of the Republic Era and developed by DSI, consists of 13 projects including 7 in the Euphrates, 6 in the Tigris Basin. 75% of energy projects and 29% of irrigation projects within the context of GAP have been realized so far (DSI 2019).

In the region, 6 irrigation schemes exist including Suruc Plain, Akçakale YAS, Şanlıurfa Harran, Yaylak Plain, Upper Harran and Bozova. These irrigation schemes are managed by water user associations (WUAs) except Suruc Plain which is operated by DSI, not included in this research due to insufficient data. The spatial distributions and locations of the irrigation schemes were given in Figure 1.

The irrigation water is supplied by Ataturk Dam which was built on Euphrates River. Akçakale Irrigation Schemes use only groundwater from 299 wells beside the river. As of 2017, a total area of 433830 ha is irrigated in the region. The annual average precipitation is 400 mm, surface and groundwater potentials are 35185 hm^3 year^{-1} and 3443 hm^3 year^{-1}, respectively (DSI 2018).

The total command area in irrigation schemes covers 195527 ha represents 45.07% of the total irrigated area in the region. Command area of Akçakale YAS, Şanlıurfa Harran, Yaylak Plain, Upper Harran and Bozova

Irrigation Schemes have 16507, 134366, 18332, 13785 and 12537 ha, respectively (Table 1). Types of irrigation water delivery systems are open channel systems in Akçakale YAS, pipelines in Şanlıurfa Harran, Yaylak Plain, Upper Harran, Bozova Irrigation Schemes. The only canalette type as well as pipelines are used in Şanlıurfa Harran scheme to deliver water to farmers. Generally, sprinkler irrigation is the preferred method to irrigate the crops in the study area except for Akçakale YAS and Şanlıurfa Harran schemes where most surface irrigation methods are used. Bozova Irrigation Scheme is the only one in which drip irrigation is the usual method. The main crops are cotton and cereals in the region (Table 1).

Table 1- Main characteristics of the irrigation schemes

Irrigation scheme	*Command area (ha)*	*Water diversion*	*Irrigation method used*	*Main Crops*
Akçakale YAS	16507	gravity pumped	Surface (100%)	Cereals (55%), Cotton (45%)
Şanlıurfa Harran	134366	gravity	Surface (100%)	Cotton (87%), Cereals (12%)
Yaylak Plain	18332	pumped	Sprinkler (100%)	Cotton (68%), Peanut (11%)
Upper Harran	13785	gravity	Sprinkler (100%)	Cotton (81%), Peanut (18%)
Bozova	12537	gravity pumped	Sprinkler (97%) Drip (3%)	Cereals (50%), Cotton (27%)

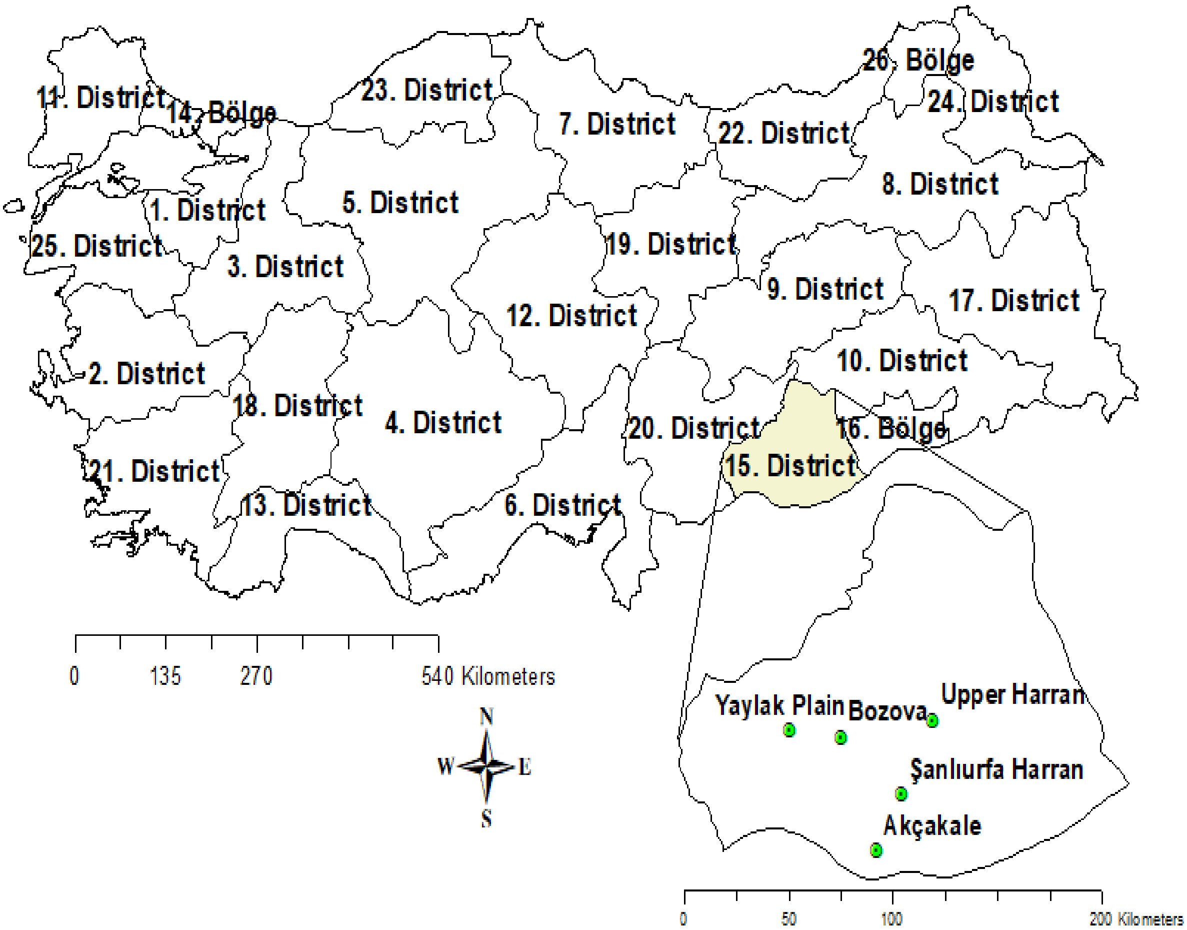

Figure 1- Spatial distribution and location of the irrigation schemes assessed

2.2. Data collection

The data between 2006 and 2016 were obtained from Monitoring and Evaluation Reports of General Directorate of DSI. The reports including command area, irrigated area, irrigation water supplied to users, irrigation water requirements, and agricultural productions are released yearly by DSI.

2.3. Calculation of performance indicators

Comparison indicators for performance were used for assessment of irrigation schemes with data from 2006 to 2016. A set of indicators required for performance comparison were chosen based on data availability and appropriateness. Performance indicators used in this study and their calculation formulas were given in Table 2.

Table 2- Performance indicators and their calculation formulas (Molden et al 1998; Burt 2001; Malano & Burton 2001)

Performance indicators	*Code*	*Formula*
Irrigation ratio (%)	I_{ratio}	$\frac{Irrigated\ area * 100}{Command\ area}$
Irrigation water supplied to users per unit irrigated area ($m^3\ ha^{-1}$)	$W_{irrigated}$	$\frac{Irrigation\ water\ supplied\ to\ users}{Irrigated\ area}$
Irrigation water supplied to users per unit command area ($m^3\ ha^{-1}$)	$W_{command}$	$\frac{Irrigation\ water\ supplied\ to\ users}{Command\ area}$
Relative water supply	R_{ws}	$\frac{Irrigation\ water\ supplied\ to\ users}{Total\ irrigation\ water\ requirement}$
Operation, maintenance and management cost per unit command area ($ ha^{-1})	$E_{command}$	$\frac{Operation,\ maintenance\ and\ management\ cost}{Command\ area}$
Operation, maintenance and management cost per unit irrigation water supplied to users ($ ha^{-1})	E_{water}	$\frac{Operation,\ maintenance\ and\ management\ cost}{Irrigation\ water\ supplied\ to\ users}$
Output per unit irrigated area ($ ha^{-1})	$O_{irrigated}$	$\frac{Output}{Irrigated\ area}$
Output per unit irrigation water supplied to users ($ m^{-3})	O_{water}	$\frac{Output}{Irrigation\ water\ supplied\ to\ users}$

Command area is the total area in the irrigation scheme, where irrigation service can be given. Irrigated area represents the irrigated area including the after crop area. Irrigation water supplied to users is the amount of water taken from the reservoir. The total irrigation water requirement is the amount of water, calculated by CROPWAT considering water delivery and field irrigation efficiency according to crop pattern (Molden et al 1998). The output is the agricultural production obtained from the irrigated area. The output changed into the American dollar ($) from local currency (Turkish lira) divided average value of the dollar related year with data of Turkish Central Bank.

2.4. Statistical evaluation

The analysis of variance (ANOVA, ANalysis Of VAriance) was used to test the difference of the performance indicators among irrigation schemes according to Ozdamar (2017). The hypothesis was established as below:

H_0: There is no difference between the performance indicators' averages.
H_1: There is a difference between the performance indicators' averages.

The correlation was investigated among indicators to determine performance indicators correlated. Shortly, correlations represent the relationships between each estimation variables and the dependent variables by controlling the effects of the other variables. Performance indicators which are correlated the others were chosen to put into the model below according in multiple linear regression analysis. The core aim of the regression model is to explain performance indicators with the others (Alpar 2017). Multiple regression model can be written as:

$$PI = cons + \beta_1 PI_1 + \beta_2 PI_2 + \cdots + \beta_i PI_i$$

Where; PI, performance indicator; β_i, coefficient of the first estimation variable I; PI_i, explanatory variable for the ith observation (dependent PI).

3. Results and Discussion

Descriptive statistics were given in Table 3 to provide general information about the district considering all irrigation schemes during the study period. The high rate of standard deviation shows irrigation water supplied to users per unit irrigated and command area were profoundly changed among the study years. This illustrates they are not reliable during the years. The other remarkable result is the leap of relative water supply (R_{ws}) as high as 7.57. Irrigation water supplied to the farmers was approximately more than twice the irrigation water requirement in the region during the period of the study.

Table 3- Descriptive statistics of performance indicators for all irrigation schemes in the study

Performance indicators	*Minimum*	*Maximum*	*Mean*	*Std. deviation*
I_{ratio}	40.96	91.91	72.18	14.89
$W_{irrigated}$	3320.71	34255.51	14105.18	7018.42
$W_{command}$	1859.82	27699.74	10652.20	6437.77
R_{ws}	0.58	7.57	2.38	1.55
$E_{command}$	31.00	2161.95	941.00	642.26
E_{water}	0.01	0.32	0.09	0.08
$O_{irrigated}$	2225.75	7961.57	4383.42	1083.19
O_{water}	0.14	1.25	0.39	0.23

I_{ratio}, irrigation ratio; $W_{irrigated}$, irrigation water supplied to users per unit irrigated area; $W_{command}$, irrigation water supplied to users per unit command area; R_{ws}, relative water supply; $E_{command}$, operation, maintenance and management cost (total expenses) per unit command area; E_{water}, operation, maintenance and management cost per unit irrigation water supplied to users; $O_{irrigated}$, output per unit irrigated area; O_{water}, output per unit irrigation water supplied to users

According to ANOVA results which were given in Table 4, there were differences in terms of irrigation ratios' averages among irrigation schemes. Irrigation ratio (I_{ratio}) showed the similarity between Akçakale and Bozova, and among Şanlıurfa-Harran, Yaylak Plain and Upper Harran Irrigation Schemes. Low irrigation ratio (57.64%) in Akçakale Irrigation Scheme may be attributed to inadequate irrigation facilities (65%), social and economic problems (17%), no irrigation water demand (15%) and the other problems (3%) according to DSI (2017). Improvement is needed in management and the farm level in Akçakale to increase irrigation ratio. Beside this, groundwater is the primary water source in Akçakale scheme and the main delivery system is open channel with low irrigation efficiency (85%). Akçakale had the lowest value of irrigation water supplied to users per unit irrigated area (8125.06 m^3 ha^{-1}) while Upper Harran had the highest value with 25035.04 m^3 ha^{-1} and there was a significant difference of irrigation water supplied to users per unit irrigated area between irrigation schemes ($P<0.001$). Although surface irrigation methods were used throughout the irrigated area in Akçakale while sprinkler irrigation method are used in Upper Harran which shows the lowest performance in terms of I_{ratio}. We can conclude that the management, maintenance and operation problems may exist in Upper Harran. Assessment of irrigation schemes with RAP (Rapid Appraisal Process) may help to determine more clearly such kind of problems (Burt 2001). Values of irrigation water supplied to users per unit command area ($W_{command}$) shows the situation of the irrigation scheme in case all service area is irrigated. In Akçakale Irrigation Scheme, $W_{command}$ was not sufficient (4930.58 m^3 ha^{-1}) compared to the irrigation water requirement (5537.40 m^3 ha^{-1}) calculated by DSI (2017) in case the command area is irrigated totally. The average relative water supply of Akçakale, Şanlıurfa Harran, Yaylak Plain, Upper Harran and Bozova were 1.68, 2.25, 1.70, 4.55 and 1.67, respectively. Irrigation water was used more efficiently in Akçakale, Yaylak Plain and Bozova than the other irrigation schemes and there were no significant differences between the averages of relative water supply. Şanlıurfa Harran and Upper Harran used water inefficiently which was more than twice the water requirement. In a similar study carried out by (Kartal 2018), Akçakale scheme was found as the most successful irrigation scheme in the district with respect to 16 performance indicators. Excessive water use in the region was also reported in his study. In the study carried out by (Corcoles et al 2010), $W_{command}$ was found between 5200 and 6800 m^3 ha^{-1} in Castilla-La Mancha where sprinkler and drip irrigation widely used. The values obtained from our study are were higher than those of their study.

It is determined that $E_{command}$ values statistically similar among 3 groups. Akçakale Irrigation Scheme formed the first group with the lowest average value, Şanlıurfa Harran and Yaylak Plain formed the second group while Upper Harran and Bozova formed the third group which have the highest values (Table 4). The high value of $E_{command}$ don't show high performance of the irrigation schemes but it may explain management, operation and maintenance requirement. The averages of total expenses per unit irrigation water supplied to users (E_{water}) were significantly different among the irrigation schemes (P<0.001) except between Şanlıurfa Harran and Upper Harran irrigation schemes (P>0.05). In this circumstance, we may conclude that management, operation and maintenance requirements were varied among the irrigation schemes in the district. Akçakale has the lowest value of E_{water} (0.01 $ m^{-3}) while Bozova the highest (0.23 $ m^{-3}). However, high expenses on irrigation scheme do not show necessarily low or high performance, but they may show the expenses to give healthy irrigation service (Alcon et al 2017).

Table 4- ANOVA results of the performance indicators between irrigation schemes

	Average values of performance indicators				
Performance indicators	*Akçakale YAS*	*Şanlıurfa Harran*	*Yaylak Plain*	*Upper Harran*	*Bozova*
I_{ratio}	57.63^{d}	79.70^{ab}	69.62^{cb}	86.47^{a}	67.46^{cd}
$W_{irrigated}$	8125.06^{c}	14550.28^{b}	11934.85^{bc}	25035.04^{a}	10880.65^{bc}
$W_{command}$	4930.58^{d}	11608.90^{b}	8172.18^{c}	21484.63^{a}	70654.7^{cd}
R_{ws}	1.68^{b}	2.25^{b}	1.70^{b}	4.55^{a}	1.67^{b}
$E_{command}$	69.06^{c}	742.72^{b}	848.96^{b}	1382.00^{a}	1662.26^{a}
E_{water}	0.01^{d}	0.06^{c}	0.10^{b}	0.06^{c}	0.23^{a}
$O_{irrigated}$	3405.84^{d}	3922.60^{cd}	5003.83^{ab}	4292.14^{bc}	5292.66^{a}
O_{water}	0.60^{a}	0.27^{bc}	0.43^{ab}	0.17^{c}	0.50^{a}

The correlation was investigated to determine the statistical relation among performance indicator and Pearson correlation result is given in Table 5. However the main aim of the correlation was to use the performance indicators as explanatory to the other in the regression model (Table 6). Since the irrigated area is close to the command area in terms of size, the highest correlation was found between $W_{irrigated}$ and $W_{command}$.

Table 5- Correlations among performance indicators

Performance indicators	I_{ratio}	$W_{irrigated}$	$W_{command}$	R_{ws}	$E_{command}$	E_{water}	$O_{irrigated}$	O_{water}
I_{ratio}	1	0.461**	0.653**	0.358*	0.375*	-0.027	0.031	-0.443**
$W_{irrigated}$		1	0.962**	0.873**	0.276	-0.206	0.014	-0.764**
$W_{command}$			1	0.825**	0.308*	-0.234	-0.009	-0.726**
R_{ws}				1	0.076	-0.264	-0.067	-0.637**
$E_{command}$					1	0.766**	0.501**	-0.194
E_{water}						1	0.582**	0.180
$O_{irrigated}$							1	0.289
O_{water}								1

**, correlation is significant at the 0.01 level (2-tailed); *, correlation is significant at the 0.05 level (2-tailed); P. corr. pearson correlation

Table 6- Multiple linear regression models

Indicators	*Model*	R^2	*P value*
I_{ratio}	*Iratio* = 84.15 - 0.005 x *Wirrigated* + 0.07 x *Wcommand* - 18.26 x *Owater*	0.84	0.000
$W_{irrigated}$	*Wirrigated* = 11979.69 - 121.13 x *Iratio* + 1.06 x *Wcommand* + 401.78 x *Rws* - 3474.21 x *Owater*	0.98	0.000
$W_{command}$	*Wcommand* = -3875.97 + 137.96 x *Iratio* + 2400.63 x *Rws* + 1.07 x *Ecommand* - 5369.10 x *Owater*	0.86	0.000
R_{ws}	*Rws* = -0.36 - 0.005 x *Iratio* + 0. 414 x *Owater*	0.77	0.000
$E_{command}$	*Ecommand* = -537.47 + 4.80 x *Iratio* + 0.04 x *Wcommand* + 6938.76 x *Ewater* - 0.01 x *Oirrigated*	0.84	0.000
E_{water}	*Ewater* = -0.06 + 8x10^{-5} x *Ecommand* + 1.9x10^{-5} x *Oirrigated*	0.64	0.000
$O_{irrigated}$	*Oirrigated* = 3538.20 + 0.23 x *Ecommand* + 6398.59 x *Ewater*	0.35	0.000
O_{water}	*Owater* = 1.44 - 0.009 x *Iratio* – 8.021x10^{-5} x W*irrigated* + 6.81x10^{-5} x W*command* + 0.02 x *Rws*	0.66	0.000

The highest average $O_{irrigated}$ was found as 5292.66 \$ ha^{-1} in Bozova Irrigation Scheme while the lowest value (3404.84 \$ ha^{-1}) was seen in Akçakale. Yaylak Plain has the highest value of average output per unit irrigation water supplied to users (0.44 \$ m^{-3}) while Upper Harran has the lowest value (0.18 \$ m^{-3}). Bozova and Akçakale Irrigation Schemes' crop patterns were similar which is the main reason increasing the output. Nevertheless, the output (production) obtained from per unit area depends on factors such as irrigation method used, farmers' experience and fertilization. We may conclude that $O_{irrigated}$ was highly changed between Bozova and Akçakale due to these parameters (irrigation methods, farmer' experience, fertilization etc.). The crop pattern of Yaylak Plain Irrigation Scheme consists of cotton (68%) and peanut (11%) while Upper Harran' crop pattern consists of cotton (81%) and peanut (18%) as main crops. Under the circumstances, Upper Harran should have higher values of $O_{irrigated}$ than Yaylak Plain Irrigation Scheme with regard to the crop pattern. This situation may be explained by internal problems as well. As it was reported in a study from Turkey (Degirmenci 2001), irrigation schemes were divided into three groups in terms of the command area. In the first group, the value of output per unit irrigated area was 1000-2000 \$ ha^{-1} in 66 irrigation schemes, 2000-3000 \$ ha^{-1} in 40 irrigation schemes in the second, and more than 3000 \$ ha^{-1} in 38 irrigation schemes in the last group. Irrigation schemes in the region fall within the third group.

The models showed that $W_{irrigated}$ can be explained with I_{ratio}, $W_{command}$, R_{ws} and O_{water}. This explains $W_{irrigated}$ was affected the most by the other indicators. We can conclude the interdependence of performance indicators from models. $W_{irrigated}$ was the performance indicator having the strongest relation correlation with others (R^2= 0.98). The lowest value of R^2 was found in $O_{irrigated}$. In this context, the models for the performance indicators with high R^2 can be used to estimate unknown indicators for the region.

When we consider the overall assessment of the study, we may conclude that $W_{irrigated}$ was exceptionally high which may originate from irrigation water management problems. Applying modernization procedures in irrigation schemes may help to use irrigation water efficiently and irrigation performance may be enhanced (Playan & Mateos 2006; Renault et al 2007; Lecina et al 2010). Although water user associations in Turkey were operated by a president who is one of the farmer-elected by other farmers' votes, DSI currently decided to assign an engineer in WUAs management. In this context, this action may be interpreted as a rational process of Turkey government to resolve problems of water management and to encourage farmers in adopting modern irrigation methods. However, agricultural extension and consultancy services to farmers are of relevant importance in increasing water use efficiency and agricultural production (Gumus & Kaya 2014).

In the current study, we figure out some performance indicators that can be explained by the other indicators according to multiple regression which similar method with Alcon et al (2017). They used panel data regression models to demonstrate the effects of Irrigation Communities' attributes such as water performance, rotation schedule, and energy prices on performance indicators in the arid region of Spain. One another study conducted by Zema et al (2015) in Italy, it was stated that there was a strong correlation between performance indicators, was also support the study of (Rodriguez-Diaz et al 2004) who ranked WUAs with factor analysis. Irrigation water was efficiently used in the arid and water shortage regions where above-mentioned studies were carried out. However, we met weak performance of irrigation schemes and inefficient water use in our study area which is also located in the arid part of Turkey.

4. Conclusions

The irrigation schemes evaluated have differences or similarities in terms of the average performance indicators in the district for the study years. Despite the fact that sprinkler irrigation is a predominant method in the application of water and the pipeline systems are mostly involved in water delivery in the district, irrigation water was mostly overused in the schemes and the agricultural production per unit irrigated area, irrigation water was low in most of the cases. Therefore, operation, maintenance and management of irrigation schemes should be reviewed and improved to eliminate inefficiency. In the region, output per unit irrigated area was found low although the crop pattern with high production values. Thus, agricultural extension and consultancy services should be enlarged to increase agricultural production.

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The Influence of Irrigation Water Salinity and Humic Acid on Nutrient Contents of Onion (*Allium cepa* L.)

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ABSTRACT
Humic acid (HA) efficiently enhances the uptake of nutrients of plants, especially on saline soil. In this study, some nutrient contents of onion in response to salinity and HA application were investigated, and effects of HA application on salinity resistance was evaluated. Research plots were established as a randomized factorial design with four replications on a lysimeter and each replication included 10 plants. Plants in the lysimeters were irrigated with tap water (control, EC: 0.3 dS m^{-1}) and four different doses of salinized water (EC: 2.0, 4.0, 6.0 and 8.0 dS m^{-1}). The HA (0 and 1.0 g kg^{-1}) was applied to the soil and mixed with the soil before planting. Increasing the levels of irrigation salinity decreased contents of K, Ca, N, P, Mg, Fe, Zn, Cu and B in onion bulbs; increased contents of Na, Cl and Mn. However, the highest content of K, Ca, and N in the bulbs were obtained by HA application under different salinity levels. Similarly, the soil application of HA positively was affected the P, Mg, Fe, Zn, B contents of the bulbs. While contents of Na, Mn, and Cu were not affected by soil application, Cl was decreased. The results showed that application of HA could partially reduce the harmful effects of salt, so HA can be used as an alternative method to improve product performance in saline conditions.

Keywords: Quality; Mineral nutrient; Onion; Salinity

1. Introduction

Since the excess uptake of toxic ions occurs in salt impacted soils, reduce in plant growth and yield may observed in most cases (Grattan & Grieve 1999). Ions such as Na, Mg, Ca, Cl, HCO_3, SO_4, and B in high quantity are responsible for soil salinity and plant growth reduction. The effect of physiological drought is one of the essential implications of salinity in plants. Soil productivity is negatively affected by a decrease in nutrient absorption of plants due to high salt concentrations in the soil (Aşık et al 2009; Khaled & Fawy 2011).

Salinity may be originated from irrigation water (Vallejo et al 2003). Limitation in the availability of water with reasonable quality water in most arid and semi-arid places entails the saline water use for agricultural production in developing countries (Bekheet et al 2006; Kiremit & Arslan 2016). Many researches were conducted to examine the effect of different irrigation water salinity on yield, growth and nutrient contents of numerous crops (Semiz et al 2012; Neocleous et al 2014).

Onion ranks third after potato and tomato in terms of crop production areas in the world. There are several problems in onion production, one of which, in the arid and semi-arid regions, is the effect of salinity. Hancı

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& Cebeci (2018) reported that onion was moderately sensitive to salinity. Initial yield decrease started at a threshold EC of 1.4 dS m^{-1}, and 50% yield reduction occurred at 4.1 dS m^{-1} (Grattan & Grieve 1999). Seed germination, seedling emergence, and seedling growth of onions were affected by soil property, the salinity of irrigation water, and irrigation method (Miyamoto et al 2010).

Humic substances are the subjects of studies in various areas of agriculture such as soil chemistry, fertility, plant physiology, and these materials can significantly improve plant growth and nutrient uptake (Paksoy et al 2010). According to Akıncı et al (2009), HA is a product contains many elements that develop the soil fertility, increase the availability of nutrient elements by holding them on mineral surfaces, and consequently affect plant growth and yield. Khaled & Fawy (2011) were reported that HA has positive impacts on the chemical, biological and physical properties of soils and nutrient uptake. Commercial products containing HA are mostly used in vegetable production; these products are applied to the soil along with liquid fertilizer (Hartz & Bottoms 2010).

The agricultural area affected by soil salinization necessitates some measures such as determination of the proper salt tolerance crop types or application of some materials to diminish the effect of salinity (Lynch & Lauchli 1988). As shown in some studies, there are positive correlations between HA and soil salinity. In this respect, Liu & Cooper (2002) said that treatments of HA might improve the plant response to the salinity since it may increase the absorption of some nutrients and decrease the uptake of some toxic substances. Kulikova et al (2005) also declared that HA might show anti-stress effects under abiotic stress conditions. Liu & Cooper (2002) also stated that more research should be carried out associated with HA application and its impacts on plant tolerance to salinity. Therefore, the present study was aimed to determine the effects of HA on the contents of some nutrients of onion grown under different salinity levels.

2. Material and Methods

2.1. Experimental site and biological material

The experiment was carried out in a greenhouse at the Mustafakemalpasa Vocational School (the coordinates are 40°02′ N, longitude 28°23′ E; and the altitude is 22 m above mean sea level), and the Department of Soil Sciences (laboratory), Uludag University, Turkey, during the spring and summer of 2013. *Allium cepa* L. cv. Banko was used in the experiment as the plant material. The onion seeds were obtained from the Unigen Seeds® (United Genetics Seeds Company, Inc., Mustafakemalpasa, Bursa, Turkey).

2.2. Treatments

This study was conducted in a completely randomized factorial design with two soil application doses of HA, (0 and 1.0 g kg^{-1}) and five levels of irrigation water salinity (EC) 0.3 (control, tap water), 2.0, 4.0, 6.0 and 8.0 dS m^{-1}. Each application consisted of four replications. There were ten plants in each lysimeter, with one lysimeter in each replicate.

2.3. Agronomic practices

During the first two weeks, all the treatments were irrigated with tap water. Irrigation treatments with saline water were imposed after two weeks from the planting date. Four salinity levels were prepared by adding a mixture of NaCl for watering the seedlings. It was necessary to maintain the quantity of the drainage water at 30-40% of the amount of nutrient solution applied; the excess water was drained through the holes in the base of the lysimeter. Humic acid obtained from liquid Deltahumate derived from leonardite (Delta Chemicals Inc., USA) was added to the soil and mixed with the soil before planting (Asık et al 2009). Seedlings of the onion cultivar were transplanted in metallic lysimeters (10 cm apart in rows of 25 cm in width) after 60 days from sowing in early March. Each lysimeter area was 0.26 m^2, 0.60 m depth and consisted of 10 onion plants. All of the lysimeters were randomized on benches in an unheated. Air-dried soil samples sieved through a 2 mm sieve and blended for homogeneity. Each lysimeter filled by 185 kg of soil. Some physical and chemical properties of the soil used for the experiments are presented in Table 1. Total of 140 kg ha^{-1} N (as ammonium sulfate) and 60 kg ha^{-1} P_2O_5 (as superphosphate) fertilizers were applied to the soil according to recommendations based on soil analyses. The amounts of mineral fertilizers were divided into two equal portions. The first portion was applied during soil preparation and the second at 60 days after onion planting. The plants were grown in a greenhouse with a day/night average temperature of 21 ºC and an average relative humidity of 70%.

2.4. Harvest and analyses

Maturity was generally observed when the crop leaves turned pale green. This was later followed by wilting and leaf fall. Whole plants were harvested in mid-July. The onion bulbs were then harvested carefully by digging them out using a hand shovel. The onion bulbs were rinsed under a tap and deionized water then sliced into pieces.

Table 1- Some physical and chemical properties of soils used in the study

Property	*Value*	*Method*	*Reference*
Bulk density (g cm^{-3})	1.44	Core	Rahimi et al (2011)
Texture (sand, silt, clay %)	29.10-48.20-24.70	Hydrometer	Soil (1951)
Organic matter (%)	1.90	Walkley-Black	Nelson & Sommers (1982)
pH (saturation)	7.80	1:2.5 water extract	Richards (1954)
EC (1:2.5 dS m^{-1})	0.45	1:2.5 water extract	Richards (1954)
Field capacity (%)	38.30	Pressure plate	Obi (1974)
Total N (%)	0.20	Kjeldahl	Bremmer (1965)
Available P_2O_5 (mg kg^{-1})	12.00	Olsen	Olsen et al (1954)
Available K_2O (mg kg^{-1})	278.00	Flame photometer	Pratt (1965)
Available Ca (mg kg^{-1})	4055.00		
Available Mg (mg kg^{-1})	578.00		
Available Fe (mg kg^{-1})	9.00	Atomic absorption spectrophotometer	Lindsay & Norvell (1978)
Available Mn (mg kg^{-1})	8.10		
Available Zn (mg kg^{-1})	0.810		
Available Cu (mg kg^{-1})	2.17		

The fresh samples were dried in a forced-air oven at 65 ºC for 48 h. Plant total N (nitrogen) was detected with a Buchi K-437/K-350 digestion/distillation unit according to the Kjeldahl method (Bremmer 1965). Total elements of plant samples were digested with HNO_3 and H_2O_2 (Berghof MWS 2 DAP 60 K microwave oven). The total cations K (potassium), Ca (calcium), P (phosphorus), Na (sodium), Mg (magnesium), Fe (iron), Mn (manganese), Zn (zinc), Cu (copper) and B (boron) were analyzed from extracts using the ICP OES (Perkin Elmer OPTIMA 2100 DV) (Kacar 2014). Cl (chloride) was determined by titration with silver nitrate using a potassium chromate indicator (Chapman & Pratt 1962). The K, Ca, N, P, Na, Cl, Mg and Fe, Mn, Zn, Cu, B concentrations in the dry matter were expressed as percentages and ppm, respectively.

The data were subjected to analyses of variance using statistical programs (IBM SPSS Statistics for Windows). Duncan is multiple range test was used to group the means of irrigation water salinity, HA, and their interactions when the F-test was significant ($P<0.05$).

3. Results and Discussion

Potassium (K) is a primary plant nutrient, which is needed by the plants in large amount. It is available to the plants in the form of cation (K^+). Potassium is essential for a variety of process photosynthesis, fruit formation, winter hardiness and disease resistance (Behairy et al 2015). In the present study, when the salinity increased, the K concentration in the onion bulbs markedly decreased under saline conditions. Our findings revealed that the interaction effect due to the combined application of salt treatments and HA application on K concentration in the onion bulb was significant as shown in Table 2. When compared with the salt only, the K content in the onion was found to be higher in the application of HA. These results were in agreement with Rauthan & Schnitzer (1981) who reported that the HA treatment raised the uptake of K, N, P, Fe, and Zn thereby improving the nutritional status of the plant.

Both Ca and K play a significant role in plant growth-development and control numerous processes. On the other hand, it is emphasized that increased salt concentrations negatively affect Ca and K intake by plant (Turhan et al 2013). In this study, compared with the control, no significant differences were found in the Ca concentration at low salinity levels (2.0 dS m^{-1}), but increasing the salt concentration of the irrigation water above 2.0 dS m^{-1} decreased the Ca content of bulbs. However, the effect of HA on the Ca level in the onion bulbs, under saline irrigation water, was significant (Table 2). The Ca content in the onion bulbs was found higher in the HA application under the saline water irrigation. In other words, the soil applications of HA may decrease the adverse effects of the increasing salinity in onion plants, and Ca play a significant role in the life of the plants, which is essential in their growth and development. According to the literature, HA stimulates roots, increases both available plant nutrients and nutrient uptake from the soil, and improves the plants' resistance to biotic and abiotic stress factors (Cimrin & Yılmaz 2005).

Table 2- The effects of salinity and humic acid on some nutrient contents in onion bulb

Treatments	*K (%)*	*Ca (%)*	*N (%)*	*P (%)*	*Na (%)*	*Cl (%)*	*Mg (%)*	*Fe* ($mg\ kg^{-1}$)	*Mn* ($mg\ kg^{-1}$)	*Zn* ($mg\ kg^{-1}$)	*Cu* ($mg\ kg^{-1}$)	*B* ($mg\ kg^{-1}$)
$NaCl_0HA_0$	2.81 b$^{(x)}$	1.40 c	2.06 d	0.61	0.29	0.25 f	0.20	41.67	19.08 cd	26.84	7.33	7.50
$NaCl_0HA_1$	2.91 a	1.90 a	2.47 b	0.66	0.28	0.25 f	0.24	41.17	21.50 bc	27.08	7.75	8.92
$NaCl_2HA_0$	2.62 c	1.38 c	2.37 c	0.62	0.36	0.29 e	0.22	40.83	25.58 a	24.87	7.83	9.25
$NaCl_2HA_1$	2.76 b	1.81 b	2.65 a	0.63	0.35	0.27 f	0.23	46.33	22.17 b	27.92	7.83	9.75
$NaCl_4HA_0$	2.13 e	1.16 ef	1.80 e	0.43	0.49	0.33 d	0.19	33.00	18.67 d	20.36	7.00	7.17
$NaCl_4HA_1$	2.48 d	1.26 d	1.69 f	0.48	0.49	0.30 e	0.22	33.67	23.42 ab	25.92	7.33	10.33
$NaCl_6HA_0$	1.79 g	1.14 f	1.37 g	0.34	0.53	0.50 b	0.17	31.50	22.75 ab	14.25	5.58	5.83
$NaCl_6HA_1$	1.86 f	1.22 de	1.31 h	0.36	0.51	0.43 c	0.19	36.58	22.42 b	16.17	5.83	7.67
$NaCl_8HA_0$	1.16 i	0.75 g	1.19 j	0.20	0.67	0.72 a	0.16	36.17	23.25 ab	11.48	5.50	5.58
$NaCl_8HA_1$	1.31 h	1.12 f	1.26 i	0.25	0.67	0.73 a	0.18	31.17	23.75 ab	11.5	6.50	7.17
NaCl ($dS\ m^{-1}$)												
0.30 (Control)	2.86 a	1.65 a	2.51 a	0.63 a	0.29 e	0.25 e	0.22 ab	41.42 a	20.29 c	26.96 a	7.54 a	8.21 a
2.0	2.69 b	1.60 a	2.26 b	0.62 a	0.36 d	0.28 d	0.23 a	43.58 a	23.88 a	26.39 a	7.83 a	9.50 a
4.0	2.30 c	1.21 b	1.74 c	0.46 b	0.49 c	0.32 c	0.21 b	33.33 bc	21.04 bc	23.14 b	7.16 ab	8.75 a
6.0	1.83 d	1.18 b	1.34 d	0.35 c	0.52 b	0.47 b	0.18 c	34. 04 b	22.58 ab	15.21 c	5.71 b	6.75 b
8.0	1.23 e	0.93 c	1.22 e	0.22 d	0.67 a	0.72 a	0.17 c	30.67 c	23.50 a	11.49 d	6.00 b	6.38 b
Humic acid (HA, $g\ kg^{-1}$)												
0.0 (Control)	2.10 b	1.17 b	1.76 b	0.44 b	0.47	0.42 a	0.19 b	35.43 b	21.87	19.56 b	6.65	7.07 b
1.0	2.26 a	1.46 a	1.87 a	0.47 a	0.46	0.32 b	0.21 a	37.78 a	22.65	21.72 a	7.05	8.77 a
NaCl	**	**	**	**	**	**	**	**	*	**	*	**
HA	**	**	**	**	ns	**	**	*	ns	*	ns	**
NaCl × HA	**	**	**	ns	ns	**	ns	ns	*	ns	ns	ns

$^{(x)}$, small and capital letters indicate significant differences among treatments; * and ** significant at the 5% and 1% of probability level, respectively; ns, non-significant

Onion plants take up a large amount of three essential nutrients, which are the basis of growth and development, such as N, P and K (Gharib et al 2016; Akhter et al 2017). Hussein et al (2015) reported a negative correlation between salinity level and N concentration. In this study, salt levels significantly influenced bulb N content. Increasing the levels of salinity were reduced the N contents. However, the interaction between salinity and N levels was significant, application of HA positively affected the N content of onion bulb. (Table 2). These results are consistent with other researchers reporting that HA increased N uptake (Eyheraguibel et al 2008; Al-Fraihat et al 2018). On the other hand, it is emphasized that increased salt concentrations negatively affect Ca and K intake (Turhan et al 2013).

In the last decade, researchers have attempted to address the problem of salinity and nutrient disorder. It is well accepted that salt-stress leads P deficiency in plants by reducing P uptake (Beltrano et al 2013). In this study, P content was highest at control and 2.0 dS m^{-1} salinity levels. P content of the onion bulbs was significantly decreased by irrigation with saline waters having the concentrations of 4.0, 6.0 and 8.0 dS m^{-1}, compared with the control (Table 2). Our findings revealed that the interaction effect between soil HA and salinity water treatments were found statistically non-significant as to K content of onion bulbs but, the soil applications of humus had a significant effect on the accumulation of P in onion bulbs. Although the applications of saline decreased the P content, the soil application of HA limited the decrease. P content gave the highest values by HA treatment. Mesut et al (2010) reported that HA enhances the uptake of some mineral nutrients by plants. The availability of phosphate and iron increased due to the humic application (Cimrin & Yılmaz 2005).

In our study, bulb Mg content was significantly influenced by NaCl levels. The Mg content did not decrease significantly until a water salinity value of 2.0 dS m^{-1} was exceeded. Increasing the levels of salinity from 2.0 dS m^{-1} to 4.0, 6.0, 8.0 dS m^{-1} significantly were reduced the Mg contents. On the other hand, the soil application of HA positively affected the Mg content of the onion plant, although the effect of the interaction of the NaCl × HA application was insignificant (Table 2). The onions treated with the application of HA rather than the untreated plants observed the higher Mg contents. The findings of this investigation are in close conformity with those of Asık et al (2009).

The results indicated that the Fe, Zn, Cu and B content of onion bulbs were strongly affected by the salt treatments; with an increased salt concentration causing a decrease in the micronutrients (Table 2). However, there were no significant differences in the Fe and Zn between the onion plants that were given the two treatments (control and 2.0 dS m^{-1}). According to the analysis results, bulb Cu and B contents increased up to 4.0 dS m^{-1}, however increasing salt doses decreased these contents significantly. Similarly, Zhu et al (2004) reported that micronutrient deficiencies were very common under plants salt stress. Mazhar et al (2012) reported that, adding different levels of HA could alleviate the harmful effect of salinity. In another study, it was reported that humic substances in saline conditions affect plant some mineral intake and increase salt tolerance (Ouni et al 2014). These results might be because humic substances are improved nutrient uptake and ability to chelate soil nutrients (Mayhew 2004). In this connection, the soil application of HA positively affected the Fe, Zn, B contents of the onion bulb, although the effect of the interaction of the NaCl × HA application was insignificant (Table 2). The higher Fe, Zn and B contents were observed by the onions treated with the use of HA rather than the untreated plants. The results obtained are supported by previous studies, as stated by Salwa (2011) that HA essentially helps the movement of micronutrients from soil to plant. The application of HA affects the uptake of Fe, Zn, and Cu plants positively (Mayhew 2004; Eyheraguibel et al 2008; Al-Fraihat et al 2018). Grown under salt stress, Mn and Cu contents of onions were not significantly affected by supplemental HA. The research finding of Osvalde et al (2012), who indicated that the different humic substances application caused no changes in onion leaf Mn concentration, also supports this outcome.

4. Conclusions

In this study, the results show that different irrigation water salinity levels significantly affected the nutrient contents in onion bulb grown in a loamy soil. Increasing the levels of irrigation salinity decreased contents of K, Ca, N, P, Mg, Fe, Zn, Cu and B in onion bulbs while increased contents of Na and Cl. Humic acid had a significant role on increase some nutrient contents (K, Ca, N, P, Mg, Fe, Zn and B) in onion grown under different salinity levels. It could be concluded that HA has a positive effect on the onion by decreasing the negative aspects of salinity stress condition. Considering the nutrient contents in the onions, the application of 1.0 g kg^{-1} soil humic acid can be recommended in loam soils of the arid and semi-arid regions where irrigation water is saline until 8 dS m^{-1}.

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The Response of Braeburn Apple to Regulated Deficit Irrigation

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ABSTRACT

Regulated deficit irrigation (RDI) is one of deficit irrigation (DI) techniques and it is developed to minimize irrigation inputs in fruit production, especially in areas where water resources are limited, is recommended for saving irrigation water in agriculture. This study was conducted to determine the effects of deficit irrigation treatments applied in different growth periods on plant water consumption, water yield relations, stomatal conductance and yield of Braeburn apple variety (grafted on M9 rootstock). Experiments were conducted in the years 2010, 2011, and 2012 at Fruit Research Institute, Eğirdir, Isparta, Turkey. Six different irrigation treatments were applied as I_1; non-deficit irrigation program, I_2; continuous deficit irrigation program (CDI), I_3; deficit irrigation program between the 40^{th} and 70^{th} days after full bloom (DAFB), I_4; deficit irrigation program between the 70^{th} and 100^{th} DAFB, I_5; deficit irrigation program between the 100^{th} and 130^{th} DAFB and I_6; deficit irrigation program between the 130^{th} and 160^{th} DAFB. The highest yield (55.2, 54.1 and 63.8 t ha^{-1} in 2010, 2011 and 2012 respectively) and water use efficiency (WUE) (0.130, 0.129 and 0.137 t ha^{-1} mm^{-1} in 2010, 2011 and 2012) values were obtained from I_3 treatment in all short-term deficit irrigation treatments. The stomatal conductance values decreased during the short-term deficit irrigation treatments, but the values increased following the deficit irrigation periods. The results revealed that apple trees grafted on M9 rootstock were influenced by short-term water stress, but they were able to cope with stress after the deficit periods. In all deficit irrigation treatments, yield response factor (Ky) ranged from 0.77 to 2.11 Apple tree yield was less sensitive to water deficit in I_3 compared to other treatments. Therefore I_3 treatment was found to be applicable in case of scarce water resources since it ensured water saving.

Keywords: Apple; Regulated deficit irrigation; Stomatal conductance; Ky

1. Introduction

The available water resources used for agricultural production in the world is decreasing, so we need to increase water-use efficiency in agricultural practices (Naor et al 2008). The highest evapotranspiration rates occur during the dry seasons in Mediterranean areas (especially in summer months), it caused severe water deficit conditions. During those months crop irrigation becomes critical for those reasons (Lo Bianco et al 2012). Many studies have been conducted on its such as partial rootzone drying (PRD), regulated deficit irrigation, whole deficit irrigation etc. (Van Hooijdonk et al 2004; Talluto et al 2008; Lo Bianco & Francaviglia 2012).

For saving irrigation water, deficit irrigation (DI) strategies are recommended without a remarkable reduction in yield for irrigated fruit trees. In fruit production, RDI is also a technique that is developed to minimize irrigation inputs, especially in areas where water resources is limited (Talluto et al 2008). A strategy to use regulated water

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deficits and reduced wetted root volume, with appropriate timing, would be useful in arid apple-growing regions to promote partitioning of metabolites that would favor fruiting in apple trees. The same information would be useful for formulating irrigation strategies when water supplies are inadequate (Ebel et al 1995). However, this approach requires clear information: 1- What are responses of fruit trees to water stress at different growing periods? and 2- Which growing periods fruit trees are less sensitive to water stress? Determining the application period of deficit irrigation is necessary for fruit trees (Fereres & Goldhamer 1990).

Turkey is one of the countries leading apple production with 3.1% of the world's apple production. Isparta, Niğde, Denizli, Karaman, and Antalya are the leading apple-growing areas. Isparta with almost 20.3% of country production is a very important apple-growing region of Turkey (TUİK 2018). The aim of this study was to determine the effects of deficit irrigation treatments on yield, water-yield relations and stomatal conductance. And also; 1) to develop deficit irrigation scheduling in order to save water against water deficit in growing seasons because of the decrease in available water resources, 2) to determine the most suitable treatment.

2. Material and Methods

2.1. Study area and plant material

The experiment was carried out at Fruit Research Institute (Eğirdir, Isparta-Turkey) for 3 years (2010-2012). The study area has a transition climate between the Mediterranean and Central Anatolia. In the study, the Braeburn apple trees grafted on M9 rootstock (planting distance 3.5 m x 1.5 m) and planted in the year 2000 were used as the plant material. Some soil characteristics of orchard soils in Table 1.

Table 1- Soil characteristics of the experimental area

Depth (cm)	*γ (g cm⁻³)*	*FC (%)*	*WP (%)*	*AWHC (mm)*	*Salinity (dS m⁻¹)*	*pH*	*Organic matter (%)*	*Texture*
0-30	1.46	24.20	11.50	55.60	0.175	8.1	1.80	Clay loam
30-60	1.38	25.10	13.10	49.70	0.125	7.9	2.70	Clay loam
60-90	1.41	24.30	12.20	51.20	0.130	8.0	2.75	Clay loam

γ, unit weight of soil. Field capacity and Wilting point at 0-90 cm soildepthare 312.7 and 156.2 mm respectively; AWHC, available water holding capacity

2.2. Irrigation treatments

A pump was used to supply the irrigation water from an irrigation canal and it was applied with drip irrigation system. Each irrigation treatment had a water meter in order to measure the irrigation water volume. Main pipe diameters, emitter spacing and flow rate were calculated according to Yıldırım (2005). For drip irrigations, two 16 mm diameter lateral pipes with in-line pressure compensated emitters were used for each tree row. Emitter spacing and discharge rate were 50 cm and 4 lh^{-1}, respectively. One mini valve was installed at each lateral input to control the irrigation water volume. Digital tensiometers were used to measure soil moisture at soil depths (30, 60, and 90 cm) (Soilspec digital tensiometer, JGK TECH, Australia) before each irrigation in each treatment and replication. One tree was selected for each replication and tensiometers were placed perpendicularly between two lateral lines under canopy of apple tree.

Six different irrigation treatments were applied as I_1; non-deficit program, I_2; continuous deficit irrigation program (CDI), I_3; deficit irrigation program between the 40th and 70th days after full bloom (DAFB), I_4; deficit irrigation program between the 70th and 100th DAFB, I_5; deficit irrigation program between the 100th and 130th DAFB and I_6 deficit irrigation program between the 130th and 160th DAFB. Full bloom dates of the Braeburn apple variety were April 21st, 20th, and 22nd in 2010, 2011, and 2012, respectively. Irrigation interval for all treatments was used as 4 days. The pan coefficient (K_p) to be used for determining the irrigation water volume to be applied was considered as 1.0 and 0.25 for I_1 and I_2 respectively during the whole vegetation period. During the deficit irrigation periods, for I_3, I_4, I_5, and I_6treatments, K_p was considered as 0.25. However, out of the deficit irrigation periods, K_p was considered as 1.0. Deficit irrigation periods were selected as 30 days due to the fact that the phenological separation of apple fruit and shoot development is not clear unlike some other fruit varieties such as pear and peach (Forshey et al 1983; Chalmers 1989). Short-term water deficit applications (I_3, I_4, I_5, and I_6) were launched after the 40th DAFB. The cell division phase of apples is effective on fruit size and lasts for 4-5 weeks after full bloom (Felmann 1996). At the end of the 4th-5th weeks after full bloom, the phase of fruit cell division is accepted to be completed. Therefore, short-term water deficit applications were initiated following these days.

2.3. Irrigation water, evapotranspiration and WUE

Irrigation water, I, was calculated in each treatment according to Equation (1) and (2) (Ertek & Kanber 2003).

$$I = E_{pan} \times K_p \times P \quad (1)$$

In Equation; I, is the amounts of irrigation water (mm); E_{pan}, the cumulative pan evaporation at 4-day irrigation interval (mm); K_p, pan coefficient; P, shaded area, 37% (0.37). Percentage of the shaded area was calculated as the ratio of the surface area shaded by trees at noon to the surface area allocated to one tree and found to be 37% (0.37). Thus, only the 37% of the total cropped area was irrigated. Evaporation was measured every day by using a Class-A pan. Calculated irrigation water according to Equation (1) was converted to total irrigation volume by using Equation (2).

$$I_v = I \times A \quad (2)$$

Where; I_v is the total irrigation water volume (liter), I is the irrigation water (mm), A is the plot area per treatment including replications (m^2). Programmed irrigation applications were initiated after the available moisture at 0-90 cm soil depth reached to field capacity at the end of the full bloom. The first and last irrigation dates were May 14th-September 27th, May 8th-September 25th, and May 14th-September 23rd, respectively for 2010, 2011, and 2012. Evapotranspiration (ET) was calculated by using soil water balance method (Equation (3)) (James 1988).

$$ET = I + R + C_r - D_p - R_f \pm \Delta s \quad (3)$$

In equation; ET, the evapotranspiration (mm); I, the irrigation water amounts (mm); R, the rainfall (mm); C_r, the capillary rise (mm); R_f is the surface run-off (mm); D_p, the water loss by deep percolation (mm); Δs, the change in profile soil water content (mm). C_r and R_f were zero, since the experimental area had not any ground water problems and emitter discharge rate was selected in accordance with infiltration rate. Precipitation was measured after every rainy day by using a pluviometer which was near the study area. Equation (4) was used to calculate water use efficiency (WUE) for all treatments (Howell et al 1990).

$$WUE = (Y / ET) \times 100 \quad (4)$$

Where; water use efficiency, WUE ($t\ ha^{-1}\ mm^{-1}$), yield, Y ($t\ ha^{-1}$), and ET is the evapotranspiration (mm).

2.4. Yield response factor

Yield response factor (k_y) of each treatment was calculated according to Equation (5) (Doorenbos et al 1986; Steduto et al 2012).

$$[1 - (Y_a / Y_m)] = k_y \times [1 - (ET_a / ET_m)] \quad (5)$$

Where; Y_a is the yield of the treatment, which k_y is calculated ($t\ ha^{-1}$), Y_m is the yield of I_1 treatment (non-deficit conditions, $t\ ha^{-1}$), ET_a is the evapotranspiration of the treatment, which k_y is calculated (mm), ET_m is the evapotranspiration of I_1 treatment (non-deficit conditions, mm), and the yield response factor is k_y.

2.5. Other measurements

Yield: Five trees were harvested in the middle of the experimental plot and all them were weighted. So the yield was determined as $t\ ha^{-1}$. Dates of harvest were October 18th, 24th, and 22nd in 2010, 2011, and 2012, respectively.

Stomatal conductance (SC): Stomatal conductance measurements were carried out on the trees with porometer (Delta-T, Porometer-AP4). Leaf samples were taken from the sun-exposed mature leaves of one year old shoots from different sides of the selected trees in every treatment. At least 5 leaves per tree were sampled, and two repetitive readings were taken from each leaf.

2.6. Experimental design and statistical analysis

Experiments were carried out in Randomized Blocks Experimental Design with three replications. Each treatment had three replications and there were two rows apple trees in each replication. Each row had 9 trees. One row in each replication was left as extra row between two replications. Only five trees in one row were considered for calculating yield and the other measurements. Therefore, all measurements and harvests were conducted on 5 trees in each replication. The analysis of variance (ANOVA) test was calculated with JUMP software (SAS Institute Inc.) for the data. The differences among treatments were compared by using LSD test.

3. Results and Discussion

3.1. Irrigation water and evapotranspiration

The total evapotranspiration and irrigation water values are given in Table 2. The highest evapotranspiration in growing period of apple occurred in 2012 because seasonal average air temperature was higher than the other years (Figure 1). The pan evaporation values in 2012 was also higher than the others (Table 2). The soil water content (SWC) measured at 90 cm soil depth before each irrigation for all treatments during the study (Figure 2). The irrigation water compensated the plant water consumption in all treatments until the 72nd DAFB, and then the soil moisture in the effective root zone started to decrease due to increasing air temperature (July and August) and rapid fruit development period (Stage II) (Atay 2007). Since the irrigation water did not compensate the plant water consumption under deficit conditions, trees consumed the available soil moisture. Similar to I_2, the soil water content decreased quickly because of the rapid fruit development stage (Stage II).

Table 2- Total evapotranspiration (ET) and irrigation water (I) of study

Treatments	*2010*		*2011*		*2012*	
	I (mm)	*ET (mm)*	*I (mm)*	*ET (mm)*	*I (mm)*	*ET (mm)*
I_1	363.8	480.8	361.2	478.0	395.0	522.7
I_2	118.4	267.4	107.8	243.9	119.3	287.7
I_3	317.1	426.2	308.7	420.7	335.0	465.9
I_4	296.5	418.3	292.4	416.9	312.6	475.8
I_5	287.3	412.8	300.8	448.2	320.2	492.9
I_6	318.7	431.9	316.6	454.8	349.3	500.6
E_{pan} (mm)	890.1		909.9		984.8	
P (mm)	80.5		87.2		114.9	

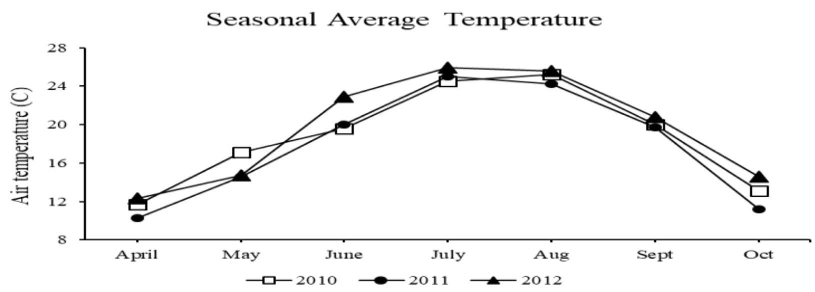

Figure 1- Seasonal average air temperature of the experimental area

Different deficit irrigation treatments were effective on the *I* and *ET* because of decreasing available water at effective root zone of apple trees (Mills et al 1997; Petillo et al 2009). Less irrigation water was applied to CDI (I_2) during vegetation period. Trees in that treatment consumed less water. The amount of plant water consumption decreased in I_3, I_4, I_5, and I_6treatments during water deficit periods. When compared to I_1, water saving ranged from 12.4 to 19.7% in 2010, from 12.4 to 18.9% in 2011, and from 11.6 to 20.9% in 2012. The highest water saving was determined in I_4 and I_5, which included deficit irrigation during the period of higher evaporation. The deficit irrigation applied between the 70^{th} and 100^{th} and between the 100^{th} and 130^{th} DAFB resulted in a decrease in the need for irrigation water in apple growing. In deficit irrigation treatments, plant water consumption decreased as the amount of irrigation water decreased. One of the periodic deficit irrigation programs mentioned in this study can be applied for water saving in growing season. However, yield should be a criterion on selection. Therefore, before mentioned factors should be taken into consideration while making suggestion to growers. In recent years, a decrease has been encountered in available water resources due to lack of precipitation and drought. Such a case is more apparent especially in the Mediterranean Basin and leads to serious water shortage (Lo Bianco et al 2012). Thus, the treatments that include water saving in growing season become even more important.

The duration of Stage I in which fruit development is slow, is 56 days after DAFB for Braeburn apple variety in Eğirdir region. Stage II is 95 days after Stage I (Atay 2007). The water content in root zone was in compliance with the fruit development rate. Roots consumed the available water in soil, and a decrease in water content was then observed in all treatments with the beginning of Stage II.

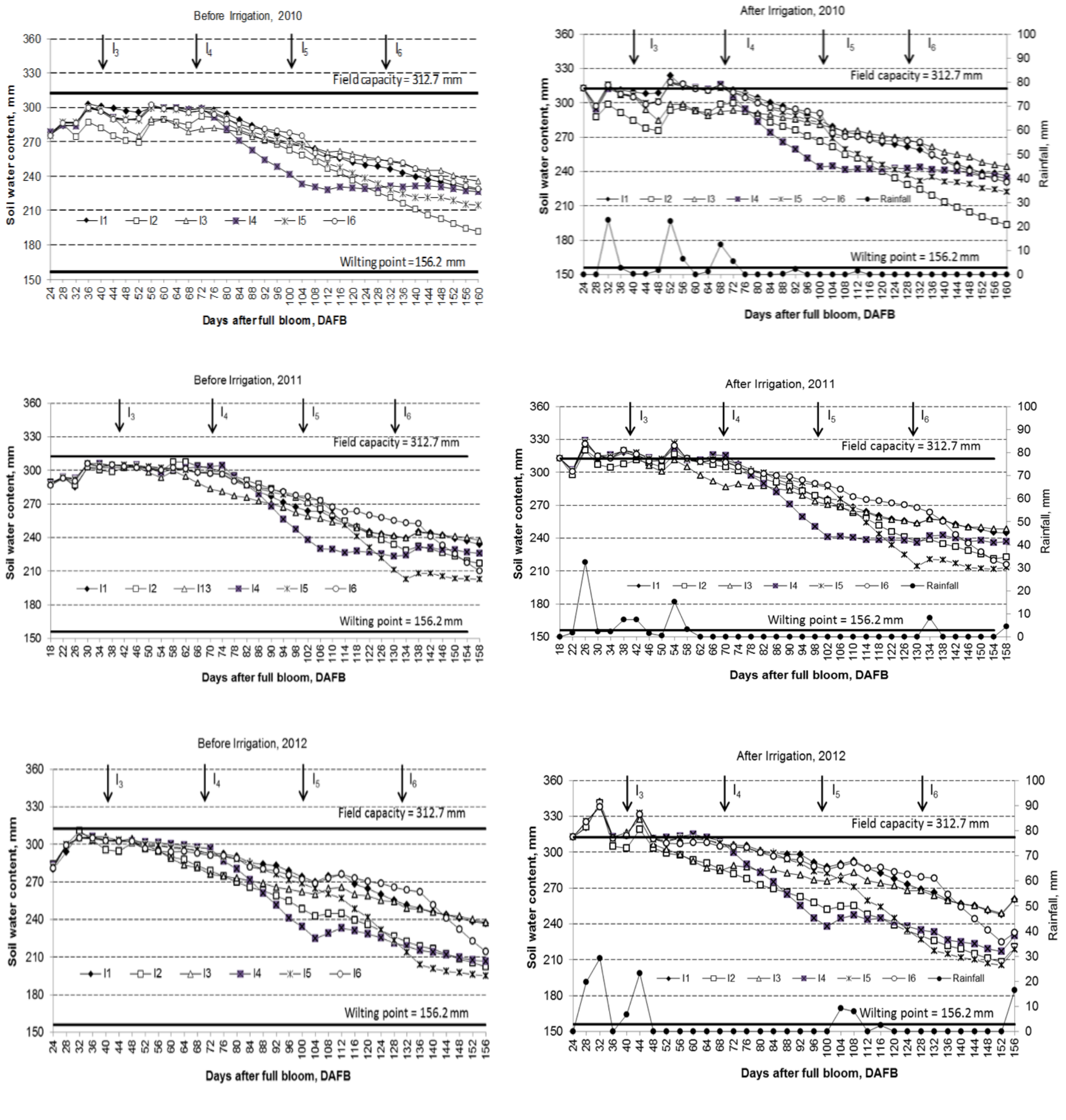

Figure 2- The soil water content at initiation and after irrigations

3.2. Stomatal conductance

Stomatal conductance (g_s) was higher in I_1 (non-deficit irrigation) but the lowest in I_2 (CDI) during the growth period (Figure 3). Stomatal conductance values in other treatments during the period of deficit irrigation were close to those of I_2. Following the end of deficit period, an evident increase in g_s values was observed. Talluto et al (2008) and Lo Bianco & Francaviglia (2012) reported that, compared to other treatments, deficit irrigation caused a decrease in g_s. Decreased g_s in DI trees often occurred as a result of soil water deficit (Mpelasoka et al 2001). Talluto et al (2008) stated that this effect should be due to relatively rapid soil drying and consequent hormonal signal. Some studies conducted on potted apples, olive and grapes have similar results with this study (Dry & Loveys 1999; De Souza et al 2003; Wahbi et al 2005). When the plant is exposed to water stress as a result of a decrease in available soil water at root zone, it closes its stomas so as to decrease evaporation from the leaf area. The closure of stomas may also occur as a result of ABAs existing in roots and produced and transferred from shoots. The closure of stomas may be regarded as a defense mechanism against drought. ABA is highly effective on the closure of stomas. Its accumulation in leaves plays a crucial role in decreasing loss of water by transpiration under stress conditions (Taiz & Zeiger 1998). In this study, ABA was not measured. However, the decrease in g_s values in water deficit periods can be explained in this way.

The difference between g_s values was significant ($P<0.01$) in two years. According to the results of g_s measurements, it could be said M9 rootstock apple trees are affected by short-term water stress but recovered from the effects of stress as soon as stress conditions ended.

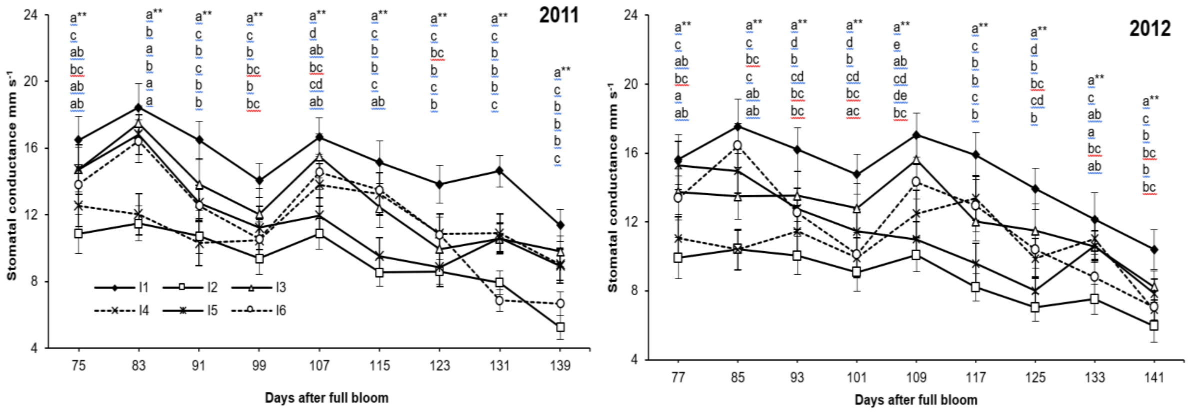

Figure 3- Stomatal conductance of treatments in 2011 and 2012 (Values with common letters do not differ significantly in each separate column (P<0.01))**

3.3. Yield

The yield obtained from 2012 was more than other years. Apple trees is very sensitive to climatic events like water stress, heat stress, heavy precipitation, and etc. during blossom period (Bekey et al 1981). The first and second years of the experiment had some climate events mentioned above. They had negative effects on fruit set and fruit numbers and the yield decreased. But climate conditions in third year was more suitable than first years of the experiment for blossom period and fruit set. That's why yield increased according to 2010 and 2011. But deficit irrigation treatments (I_3, I_4, I_5 and I_6) had less yield than I_1 as in 2010 and 2011.

The effect of treatments on yield was significant ($P<0.01$) for 3 years (Table 3). The yield was affected less in I_3 than it was in other deficit irrigation treatments. It was determined that I_3 had less negative effects on yield in this study. The yield in I_3 was closes to that in I_1 and declined by 6% and 11%. As an average over three years, a yield decrease of 10%, 18%, 18%, and 13% was observed in I_3, I_4, I_5, and I_6, respectively, compared to I_1. While CDI caused 34% and 38% reduction in yield in 2010 and 2011, it reached 55% in 2012. The transportation effect of water deficit on apple tree development and yield could be determined in subsequent years (Doorenbos et al 1986). Girona et al (2010a) also reported that higher water stress during the late development period of apple trees led to yield reduction in development process of the next year.

Table 3- The yield values obtained from treatments

Treatments	*2010*		*2011*		*2012*	
	Yield (t ha^{-1})	*Decrease in yi (%)*	*Yield (t ha^{-1})*	*Decrease in yield (%)*	*Yield (t ha^{-1})*	*Decrease in yield (%)*
I_1	58.8 a**	0.0	61.1 a**	0.0	71.7 a**	0.0
I_2	38.9 d	33.8	37.6 c	38.5	32.2 c	55.1
I_3	55.2 ab	6.1	54.1 ab	11.5	63.8 ab	11.0
I_4	50.4 bc	14.3	48.5 b	20.6	58.0 b	19.1
I_5	46.7 c	20.6	51.7 b	15.4	58.1 b	19.0
I_6	51.8 bc	11.9	53.1 b	13.1	62.1 ab	13.4

**, P<0.01, *Values with common letters do not differ significantly in each separate column*

Deficit irrigation treatments had lower yield than non-deficit treatment. It was reported that deficit irrigation applications reduced yield in apple because available water at effective root zone was less than no-deficit irrigation treatment (Mpelasoka et al 2001; Petillo et al 2009; Girona et al 2010b). Available water in soil is very important for root activity. The irrigation water applied to treatments affects the available water in soil to be used by apple trees and consequently plant water balance and yield (Naor et al 1997). Amounts of available soil water were very different because of different irrigation water amounts applied to treatments in this study. The yield of deficit irrigation treatments was less than no-deficit irrigation treatment in this study like related other studies. Apple trees grafted on M9 rootstock had shallow root system, and thus they were sensitive to soil moisture reduction (Ferre & Carlson 1987). Different yields were obtained from the treatments because different irrigation water amounts were applied in this study. The yield obtained from treatments was closely associated with the irrigation water amounts and evapotranspiration.

3.4. Water use efficiency and Ky

WUE ranged between 0.112 and 0.154 t ha^{-1} mm^{-1} during the study (Table 4).The irrigation treatment having the lowest water consumption (I_2) gave the highest results for the first two years but the lowest WUE in the third year. The reason may be that the stress conditions of the first two years affected the third year. Apart from that, the highest values were found in I_3. In other periodic deficit irrigation treatments, however, values were close to each other. The reason why the WUE values of I_4, I_5, and I_6 were lower than those of I_1 may be that the water deficit of those periods affected yield negatively. Some researchers reported a lower WUE values in commercial irrigation treatments than in deficit irrigation treatments in fruit trees (Mitchell & Chalmers 1982; Zegbe & Behboudian 2008; Girona et al 2010b). This study has a different result.

Table 4- Water use efficiency (WUE) of irrigation treatments in 2010, 2011, and 2012

Treatments	*WUE (t ha^{-1} mm^{-1})*		
	2010	*2011*	*2012*
I_1	0.122	0.128	0.137
I_2	0.145	0.154	0.112
I_3	0.130	0.129	0.137
I_4	0.120	0.116	0.122
I_5	0.113	0.115	0.118
I_6	0.120	0.117	0.124

The relationship between relative decrease in yield (1-(Y_a/Y_m)) and relative decrease in plant water consumption (1-(ET_a/ET_m)) for each treatment is shown in Figure 4. The linear equations obtained from 3 years' data for each deficit irrigation treatment were well fitted, since the r^2 values are between 0.83 and 0.99. Both the magnitude and the duration of water deficit affect plant yield directly. Under deficit water conditions, compared to higher Ky values, lower k_y values in whole or partial (independent) plant vegetation periods cause less yield losses (Doorenbos et al 1986). During the study, proportional yield loss corresponding to proportional plant water consumption was at the lowest level in the deficit irrigation treatment between the 40th and 70th days after full bloom (I_3) compared to other deficit irrigation treatments. Yield reduction in apple trees is almost directly consistent to reducing plant water consumption (k_y= 0.97) in CDI (I_2). In this study, both the magnitude and the duration (i.e. 30 days) of the deficit irrigation applications were same in I_3, I_4, I_5, and I_6. However, the yield of apple trees was found more sensitive under the stress conditions between the 70th and 100th days, between the 100th and 130th days, and between the 130th and 160th days compared to the stress conditions between the 40th and 70th days. The other reason of this, fruit growth was slower in I_3 period than the other deficit periods. Therefore fruit growth may be affected less. Crop response was very sensitive to water deficit ($k_y>1$) in I_6, I_5, and I_4, respectively. Ebel (1991) and Girona et al (2010a) also reported that higher water stress during the late development period of apple trees leads to yield reduction in the development process of the following year.

In 2010, the yield response factor (k_y) for I_2, I_3, I_4, I_5 and I_6 were, respectively, 0.85, 0.75, 1.23, 1.63, and 0.99. The values were 0.82, 0.81, 1.07, 1.21 and 1.40 in 2011. The corresponding values in 2012 were: 1.25, 0.77, 1.47, 2.11, and 2.11, respectively. K_y values of I_3 treatment were under 1.00 for three years. The increase in K_y from 2010 to 2012 was 61%, 90%, 65%, 130% and 171%, respectively in I_2, I_3, I_4, I_5 and I_6. The reason for this was; although yield and ET were higher in 2012 than 2010, decrease in yield corresponding to per unit water decrease, i.e. response of yield to decrease in evapotranspiration, was higher in 2012 than 2010.

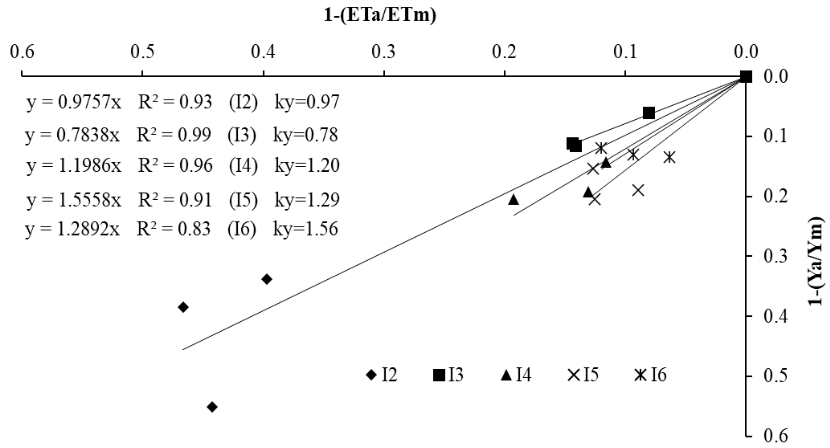

Figure 4- Ky values obtained from apple trees under deficit irrigation practices

4. Conclusions

It was obtained that the response of Braeburn apples to deficit irrigation treatments was different. Trees were affected by short-term stress but overcame stress conditions as soon as the deficit irrigation period ended. Of all deficit irrigation treatments, I_3 gave the highest yield and WUE with water saving. Decrease in the yield of I_3 with the deficit irrigation applied between the 40th and 70th DAFB was less than that of the other deficit irrigation treatments. Therefore, I_3 was found to be more suitable than all the other deficit irrigation programs when water scarcity happens since it also ensures water saving.

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Observation of Weed Species, Frequency and Density in Common Barley (*Hordeum vulgare* L.) Fields of Diyarbakir, Turkey: A Case Study

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ABSTRACT

The weed species in the common barley (*Hordeum vulgare* L.) fields in the Diyarbakir were observed with this study. For this purpose, 50 quadrats of 1m by 1m (1 m^{-2}) were randomly placed and examined at each study site. In every set of the quadrat, frequency and density were observed throughout the cropping season of 2015-2016. During the course of field study 72 weed species belonging to 21 families, 13 grasses, 59 broadleaves were recorded. The common families were Asteraceae and Poaceae in the common barley fields. The encounter frequency of weeds was determined by observing 80% wild mustard (*Sinapis arvensis* L., 80%), animated oat (*Avena sterilis* L., 63%), common wild oat (*Avena fatua* L., 54%), corn buttercup (*Ranunculus arvensis* L., 54%), corn poppy (*Papaver rhoeas* L., 52%), creeping thistle (*Cirsium arvense* (L.) Scop., 51%), volunteer lentils (*Lens culinaris* Medik., 51%). Wild mustard, which had the highest frequency was also the densest weed species (5.18 plant m^{-2}), and other species such as animated oat (4.33 plant m^{-2}), creeping thistle (1.77 plant m^{-2}), common wild oat (1.72 plant m^{-2}), corn buttercup (1.47 plant m^{-2}), cleavers (1.38 plant m^{-2}), corn poppy (1.22 plant m^{-2}), volunteer lentils (1.07 plant m^{-2}) were important species at all the observed fields. It was observed that the great infestation was shown by broadleaf weeds due to the lack of effective weed control in the barley areas.

Keywords: Common barley (*Hordeum vulgare* L.); Weed species; Abundance; Distribution; Infestation

1. Introduction

The common barley (*Hordeum vulgare* L.) is an annual grass and long-day plant belonging to the Poaceae family and grown in the cool climate temperatures of spring and winter (Awika 2011; Koehler & Wieser 2013). It is a significant grain after common wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and corn (*Zea mays* L.) as global (IGC 2018). The worldwide common barley production has been between 130 and 150 million tons from year to year. Turkey's annual production has been between 7-8 million tons (FAOSTAT 2014). Southeastern Anatolia Region of Turkey, which has 16% of the production of barley due to animal husbandry and marketing opportunities. The Diyarbakir, which is the grain center of the region, grows 2% of the country's common barley production (TUIKSTAT 2015). The barley is the principal dryland crop, and general winter planted in the region, both two-rowed (Namely; Sahin-91, Sur-93, Samyeli, Baris and Hevsel) and six-rowed (Namely; Kral-97, Vamik Hoca-98, Akhisar, Kendal and Altikat) varieties are grown (TTSM 2018). The selection of two-rowed or six-rowed barley varieties by farmers depends on the current environment, climate and diversity. Both are used to make animal feeding, malting and food making (Coken & Akman 2016; AHDB 2018). There are many abiotic and biotic factors such as temperature**,** light**,** and soil (nutrients), bugs, fungi,

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bacteria and weeds that can cause yield loss in the common barley crop. The severity of yield loss depends on water capacity of the soil, drought, frost, barley varieties, planting norm and density of diseases, pests, and the weeds (Afentouli & Eleftherohorinos 1996; Samarah 2005; Jaggard et al 2010; Schumacher et al 2018).

Although the common barley has a suffocating effect on various weeds through crop intervention (Lanning et al 1997), some weeds have the potential to reduce yield (Lyon & Young 2015). The weeds not only compete with the common barley for nutrients, water and light, but can also make crop harvesting more difficult, increase clamping, and promote insect infestation or mold growth in stored grain (Swanton et al 2015). The common barley crop that is contaminated with the weeds may not be able to reach malt species, and the taste can be reduced when used as animal feed, therefore the weeds reduce crop quality. In the common barley fields, the grass weeds such as common oat (*Avena fatua* L.), animated oat (*Avena sterilis* L.), rigid rye-grass (*Lolium rigidum* L.), and canarygrass (*Phalaris brachystachys* L.) are the most threatening to the barley production. The broadleaf weeds such as cornflower (*Centaurea cyanus* L.), knapweed (*Centaurea depressa* Bieb.), thistle [*Cirsium arvense* (L.) Scop.], field bindweed (*Convolvulus arvensis* L.), wild carrot (*Daucus carota* L.), cleavers (*Galium aparine* L.), prickly lettuce (*Lactuca serriola* L.), groundsel (*Senecio vulgaris* L.), wild mustard (*Sinapis arvensis* L.), and false carrot [*Turgenia latifolia* (L.) Hoffm.] are the most threatening species for the barley growth (Turk & Tawaha 2003; Kordali & Zengin 2011; Guncan 2014; Tepe 2014; Veisi & Moeini 2015).

Increased knowledge on how to identify and destruction of the weeds in the common barley produced areas reasonable a significant tactic for the weed control. The prevalence level and development of the abundance and dispersal of the weeds depend on weed control methods. Weed determination researches can supply valued data to growers and scientist on whether weed control is warranted, and if so, what are the favorable weed management strategies (Kumar & Jha 2017). The aim of this study is to contribute to the academic literature on weeds that have caused problems in the barley cultivated fields. In addition to the barley cultivation, the study raises awareness that weeds can pose a threat to long-term sustainable weed control. Therefore, this research was undertaken to detect the frequency and density of the weeds in the barley fields.

2. Material and Methods

2.1. Plant material and field trial

The current study deals with weed species found in the Diyarbakir common barley fields. The study was based on surveys of common barley covered area during the cropping seasons of 2015-2016. The sampling areas were selected to represent the area according to the size of the sowing area and the samples were calculated by the sectioned sampling method (Bora & Karaca 1970). Survey studies were carried out in all of the 17 districts where the common barley is grown in the Diyarbakir province. For these purposes, 180 fields of the common barley were observed across the Diyarbakir located in Southeastern Anatolia Region to find out more about weed species in the 2015-2016 cropping season (Figure 1).

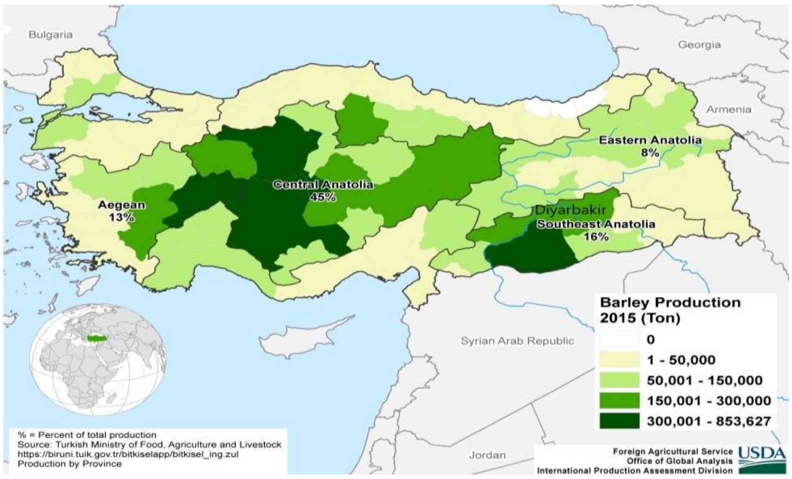

Figure 1- The location of the studied area Diyarbakir province in Turkey

As a result of this, a proportional distribution was made taking into account the maximum production areas (Table 1).

Table 1- Barley cultivation areas and sampling numbers according to the districts of Diyarbakir province

Districts	*Barley fields (da)**	*Sample number*
Baglar	30.000	10
Bismil	105.885	36
Cermik	22.194	7
Cinar	68.540	23
Cüngüs	4.996	2
Dicle	4.977	2
Egil	17.956	6
Ergani	120.000	40
Hani	17.914	6
Hazro	5.500	2
Kayapinar	9.000	3
Kocakoy	3.500	1
Kulp	39.227	13
Lice	649	1
Silvan	10.000	3
Sur	70.000	24
Yenisehir	2.971	1
Total	533.309	180

*, data are taken from TUIK (2015)

The field studies were carried out during the peak weed growth season, which is from March to May when weeds could be easily identified. Field surveys were performed twice a week to each site to collect weed species. Care has been taken to ensure that there was a minimum distance of 3 km between each field in the fields where the sample was taken from. During this research, interviews were carried out with producer and agriculturists from each field concerning weed species which are characteristic of a particular season of the year and important notes on germination, 2-6 leaves, flowering and fruiting seasons of weeds. The counts have been started within at least 10 m of the fields to remove the edge effect. Quadrats 1 m by 1 m (1 m^2) were randomly laid down in the agricultural fields to quantify various weed species, and they were used 4 times for a decare chosen to represent the field in the fields studied (Odum 1971).

2.2. Computation and data analysis

The broadleaf weeds were evaluated as whole plants and the grasses were evaluated as stalks and were reported survey forms. The vegetation structure and composition in the agricultural areas have been compared with the plant frequency and density, which is the simplest and most popular measurement methods for measuring abundance and distribution of weed species (Nkoa et al 2015). Different phytosociological parameters such as plant frequency (%) and density (plant m^{-2}), were calculated by using the following equations (Guncan 2014):

% Frequency = (Number of sampling units in which the species occurs (*N*))/(Total number of sampling units employed for the study (*Q*))*100

$$F=(N/Q)*100 \quad (1)$$

Where; *F*, *f*requency; *N*, number of quadrats in which the species is present; *Q*, *t*otal number of quadrats studied.

Density = (Total number of individuals of the species in all the sampling unit (*S*))/(Total number of sampling units studied (*Q*))

$D=(S/Q)$ (2)

Where; *D*, density; *S*, total number of individuals; *Q*, total number of quadrats studied.

The weed species that could not be diagnosed in the field were appropriately collected, pressed, dried, preserved and identified according to Davis (1965-1989). The same grading method was used to score the predominant weed species. The weeds were recorded in the field when the density of weed species was less than 20% per square meter, from 20 to 40% in medium and when the density was more than 40%.

3. Results and Discussion

As a result of observations made in the common barley fields of the Diyarbakir, there were 72 weed species belonging to 21 families; including 13 monocotyledons, and 59 dicotyledons. The overall results of common barley weeds were presented in Table 2. The main biological groups were identified: monocots and dicots, annuals, biennials, and perennials, including rhizome plants. *A. fatua*, *A. sterilis*, *C. arvense*, *G. aparine*, *L. culinaris*, *P. rhoeas*, *P. bractystachys*, *R. arvensis*, *S. arvensis*, *T. latifolia* were determined as highly spread in the common barley field. According to the results of the survey, it can be concluded that the most common weed families in the common barley fields in the region were 13 species of the Asteraceae and Poaceae. In addition, other families such as Brassicaceae, Fabaceae, Caryophyllaceae, Apiaceae, Ranunculaceae, Geraniaceae, Papaveraceae etc. was recorded, but at low levels with less than 10 weed species. The weed families identified in the study (Figure 2).

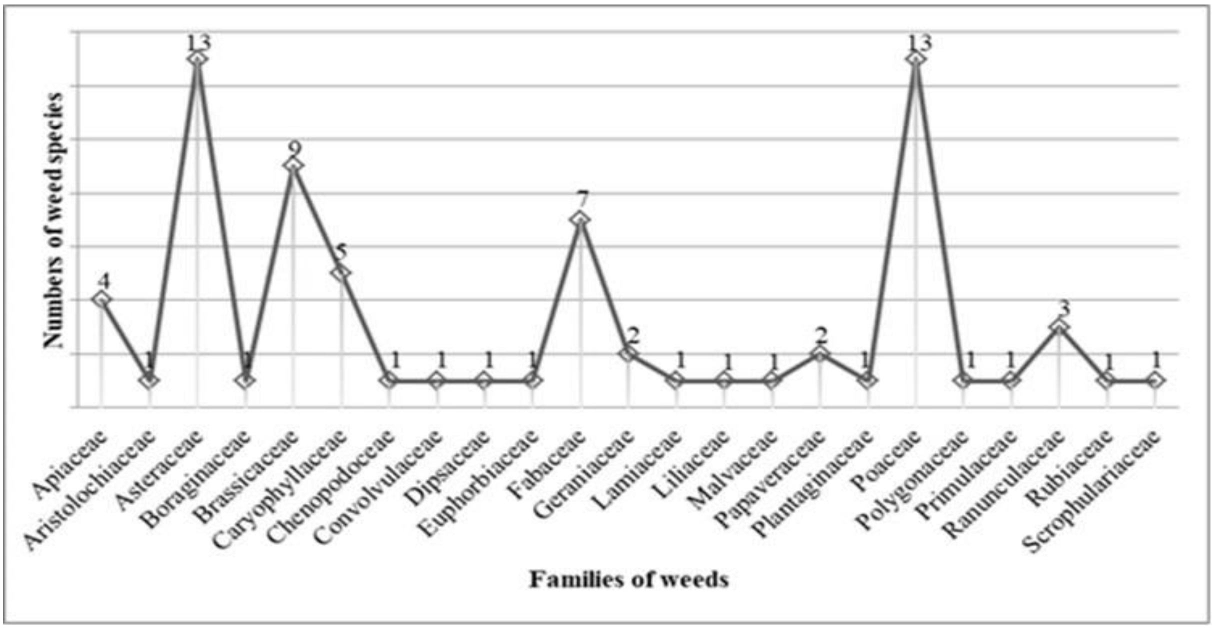

Figure 2- Families of weed species in barley cultivated areas of Diyarbakir province and the number of weed species owned by these families

The results of the surveys conducted in the Diyarbakir common barley fields showed that the frequency of the occurrence of more than 50% was *Sinapis arvensis* L. (80%), *Avena sterilis* L. (63%), *Avena fatua* L. (54%), *Ranunculus arvensis* L. (54%), *Papaver rhoeas* L. (52%), *Cirsium arvense* (L.) Scop. (51%), *Lens culinaris* Medik (51%). When evaluated according to the density of weeds; *Sinapis arvensis* L. (5.18 plant m^{-2}), *Avena sterilis* L. (4.33 plant m^{-2}), *Cirsium arvense* (L.) Scop. (1.77 plant m^{-2}), *Avena fatua* L. (1.72 plant m^{-2}), *Ranunculus arvensis* L. (1.47 plant m^{-2}), *Galium aparine* L. (1.38 plant m^{-2}), *Papaver rhoeas* L. 1.22 plant m^{-2}), *Lens culinaris* Medik (1.07 plant m^{-2}) species were found to be more than one in square meters (Table 2). It was seen that the densest and frequent species were *S. arvensis* and *A. sterilis*. However, there were some parallels in the frequency and density of weeds. For example, both the frequency and intensity of *Ranunculus arvensis* had been detected in the unit area.

Table 2- Frequency and density of weeds detected in barley fields of Diyarbakir

Scientific names of weeds	*Common names of weeds*	*Family*	*F**	*D***
Adonis aestivalis L.	Summer pheasant's eye	Ranunculaceae	5	0.27
Agrostemma githago L.	Common corncockle	Caryophyllaceae	5	0.10
Allium sp.	Onion	Liliaceae	2	0.05
Alopecurus myosuroides Huds.	Meadow foxtail	Poaceae	15	0.46
Anagallis arvensis L.	Scarlet pimpernel	Primulaceae	7	0.15
Anchusa azurea Miller.	Italian bugloss	Boraginaceae	2	0.04
Anthemis arvensis L.	Mayweed	Asteraceae	20	0.35
Aristolochia maurorum L.	Birthwort	Aristolochiaceae	8	0.15
Avena fatua L.	Common wild oat	Poaceae	54	1.72
Avena sterilis L.	Animated oat	Poaceae	63	4.33
Bifora radians Bieb.	Wild bishop	Apiaceae	25	0.45
Boreava orientalis Jaub and Spach.	Yellow weed	Brassicaceae	14	0.35
Bromus tectorum L.	Cheatgrass	Poaceae	25	0.68
Capsella bursa-pastoris (L.) Medik.	Shepherd's purse	Brassicaceae	21	0.67
Cardaria draba (L.) Desv.	Whitetop hoary cress	Brassicaceae	9	0.16
Carduus pycnocephalus L.	Italian thistle	Asteraceae	3	0.09
Caucalis platycarpos L.	Bur-parsley	Apiaceae	1	0.01
Centaurea depressa Bieb.	Cornflower	Asteraceae	16	0.46
Centaurea solstitialis L.	Yellow star-thistle	Asteraceae	13	0.34
Cephalaria syriaca (L.) Schrad.	Syrian cephalaria	Dipsaceae	6	0.15
Cerastium dichotomum L.	Mouse-ear chickweed	Caryophyllaceae	5	0.09
Chondrilla juncea L.	Rush skeletonweed	Asteraceae	14	0.35
Cichorium intybus L.	Common chicory	Asteraceae	14	0.17
Cirsium arvense (L.) Scop.	Canada thistle	Asteraceae	51	1.77
Consolida orientalis (Gay) Schrid.	Larkspur	Ranunculaceae	6	0.13
Convolvulus arvensis L.	Field bindweed	Convolvulaceae	25	0.89
Daucus carota L.	Wild carrot	Apiaceae	21	0.56
Descurainia sophia (L.) Webb	Flixweed	Brassicaceae	2	0.03
Erodium hoefftianum C.A.Mey	Redstem filaree	Geraniaceae	9	0.19
Euphorbia helioscopia L.	Sun spurge	Euphorbiaceae	3	0.07
Fumaria officinalis L.	Common fumitory	Papaveraceae	22	0.66
Galium aparine L.	Cleavers	Rubiaceae	45	1.38
Geranium dissectum L.	Cut-leaved crane's-bill	Geraniaceae	23	0.92
Hordeum murinum L.	Mouse barley	Poaceae	9	0.03
Lactuca serriola L.	Prickly lettuce	Asteraceae	22	0.65
Lamium amplexicaule L.	Henbit dead-nettle	Lamiaceae	12	0.25
Lathyrus sp.	Chickling pea	Fabaceae	4	0.08
Lens culinaris Medik	Volunteer lentil	Fabaceae	51	1.07
Lolium temulentum L. Lam.	Darnel ryegrass	Poaceae	11	0.18
Lolium rigidum L.	Wimmera ryegrass	Poaceae	27	0.38

Table 2 (Continue)- Frequency and density of weeds detected in barley fields of Diyarbakir

Scientific names of weeds	*Common names of weeds*	*Family*	*F**	*D***
Malva neglecta Wallr.	Dwarf mallow	Malvaceae	6	0.12
Matricaria chamomilla L.	Chamomile	Asteraceae	7	0.17
Medicago sativa L.	Alfalfa	Fabaceae	5	0.25
Myagrum perfoliatum L.	Musk weed	Brassicaceae	8	0.11
Neslia paniculata (L.) Devs.	Ball mustard	Brassicaceae	8	0.24
Papaver rhoeas L.	Common poppy	Papaveraceae	52	1.22
Phalaris brachystachys Link.	Short-spiked canarygrass	Poaceae	42	0.48
Phalaris canariensis L.	Canarygrass	Poaceae	25	0.29
Phragmites communis Trin.	Common reed	Poaceae	5	0.20
Pisum sativum L.	Garden pea	Fabaceae	8	0.17
Plantago lanceolata L.	Ribwort plantain	Plantaginaceae	3	0.06
Poa annua L.	Bluegrass	Poaceae	2	0.01
Poa trivialis L.	Rough bluegrass	Poaceae	26	0.42
Ranunculus arvensis L.	Corn buttercup	Ranunculaceae	54	1.47
Rumex crispus L.	Curly dock	Polygonaceae	1	0.02
Salsola kali L.	Russian thistle	Chenopodoceae	6	0.12
Secale cereale L.	Cereal rye	Poaceae	6	0.10
Senecio vulgaris L.	Groundsel	Asteraceae	5	0.08
Silene conoidea L.	Large sand catchfly	Caryophyllaceae	6	0.14
Silene vulgaris (Moench) Garcke.	Bladder campion	Caryophyllaceae	4	0.18
Silybum marianum (L.) Gaertner	Milk thistle	Asteraceae	17	0.35
Sinapis arvensis L.	Wild mustard	Brassicaceae	80	5.18
Sisymbrium officinale (L.) Scop.	Hedge mustard	Brassicaceae	9	0.24
Sonchus asper (L.) Hill.	Spiny sowthistle	Asteraceae	3	0.06
Sonchus oleraceus L.	Common sowthistle	Asteraceae	4	0.35
Thlaspi arvense L.	Field pennycress	Brassicaceae	15	0.89
Trifolium sp.	Clover	Fabaceae	4	0.13
Turgenia latifolia (L.) Hoffm.	Broadleaf false carrot	Apiaceae	41	0.96
Vaccaria pyramidata Medik	Cowherb	Caryophyllaceae	17	0.52
Veronica hederifolia L.	Ivy-leaved speedwell	Scrophulariaceae	21	0.30
Vicia faba L.	Broad bean	Fabaceae	1	0.02
Vicia sativa L.	Common vetch	Fabaceae	8	0.44

* F, frequency (%); ** D, density (plant m^{-2})

As a result of exploratory realized to detect the frequency and density of weed species seen in the common barley fields of the Diyarbakir, the numbers of species of Asteraceae and Poaceae were found to be higher in a number of species. It is a natural result that most of the weed species have emerged from these two families because the plant families are two of the families with the highest species in our city cereal fields (Pala & Mennan 2017). Previous surveys had shown that these two families are among the most species-bearing families (Kordali & Zengin 2011).

As a result of the surveys carried out, it was observed that winter weed species (*S. arvensis, A. sterilis, C. arvense, R. arvensis, G. aparine*) were problematic. It is anticipated that these species, which have been well adapted to these ecological conditions because of the cold and rainy winters and hot and dry summers in the Diyarbakir, are expected to be a problem in grain fields (Zel 1974; Uludağ 1997; Sizer & Tepe 2016; Pala & Mennan 2017). This work is very important because it contains data that will form the basis of weed control work to be done in the common barley.

Climate, agricultural activities, and especially applied the weed control methods can change the weed composition in agricultural areas. Ergani, Bismil, Sur and Cinar districts of the Diyarbakir province were found to be heavily weeded in monoculture farming areas where dry farming was done. Crop rotation decreased weed species (Pala et al 2018), but it wasn't enough alone. Hence there was no obvious effect on the weed flora these districts.

Identification of weed distribution can be a significant point in the weed control in the common barley production. Monitoring weeds in the studied areas will help to define the implementation of appropriate management preventions (Moeini et al 2008). Veisi & Moeini (2015) found that *C. arvense* and *C. depressa* species, also *Avena*, *Galium*, and *Vicia*, a genus which is the prominent weeds in Kermanshah common barley areas, observed in the Diyarbakir too, this indicates that these weeds can be a trouble in different ecosystems. The very limited study had been done on weeds which are a problem in the common barley fields, both in Turkey and the world. Therefore, there are limited studies to compare weed species in Diyarbakir barley fields with weeds in other barley cultivation areas. The weed species found by Kordali & Zengin (2011) in the common barley fields of Bayburt differ significantly in terms of species and density compared to the weeds we have identified in our study. This explains the frequency and density of the weed species, density, and coverage areas vary in different regions and years. Various factors such as early planting, densely sown and appropriately fertilized can apply to enhance crop yield in common barley in small agricultural systems of semi-arid regions and reduce weed populations (Elwis & Young 2000; Poggio 2005). Santin-Montanya et al (2013) reported that the application of new farming techniques led to constant changes in the weed population, while some strains of some weeds lost some of the previously no problematic species. On the other hand, Schumacher et al (2018) note that the loss of weed biodiversity in agricultural fields is a global issue that should be avoided to protect the supported ecosystem services and food networks. The weed flora of the product varies from field to field depending on the environment terms, irrigation systems, fertilization, soil structure and weed management (Anderson & Beck 2017). Inadequate weed management in the Diyarbakir common barley fields and incomplete and faulty applications are increasing the weed problem in these areas. It was observed that chemicals were used to control *Avena spp.* and *Sinapis arvensis*, but these weeds could be partially controlled. The results showed that preventive and cultural methods such as certified seed use, development of tolerant variety, deep tillage with the pre-sowing disc, frequent sowing, late sowing should be developed because the current weed control methods are not sufficient. On the other hand, attention should be paid to the avoidance of early and late applications in the fertilization of common barley with the herbicide, the spraying of a well-calibrated sprayer in the appropriate climatic and soil conditions during the 2-6 leaf period of the weeds. In recent years, due to increased resistance to herbicides in the weeds of cereals in the Diyarbakir (Sizer & Tepe 2016), the rotation of the herbicide is an important consideration to be taken to reduce the weed populations. Awareness activities of the common barley producers need to be done on the weeds.

4. Conclusions

The Diyarbakir is one of the common barley gene and production center in Turkey. Several researchers have tried to explore of the weeds, but the studies on species diversity of weeds in the common barley are still unscreened. Hence, in the present study attempts were made to screen out the structure of weed communities associated with the common barley crop. This study provides preliminary data of the different categories of weeds in the common barley crop fields. According to the results of the survey, it can be concluded that the common barley plants in the region are affected by a series of the weeds. It was found that the broadleaf weeds were significant, especially *Sinapis arvensis* and *Ranunculus arvensis* were dense in the common barley which is known to have highly competitive with weeds. Consequently, the weeds were a serious concern in the common barley fields, in particular, the winter weeds were a serious problem in the common barley fields, hence it ought to seek new solutions for controls. The study is helpful to farmers, agronomists, and researchers related to this field for identification of weeds and to understand the distribution and growth patterns of weeds associated with the common barley fields. During the study, it became obvious that farmers should be trained in suitable common barley crop management practices and need to make better access to advanced weed management practices. New varieties that are tolerant to the climatic factors and herbicides, resistant to diseases and insects and that have strong weed competition, meet production and consumer’s needs should continue to grow. Weed identification and control should be taken critically. It is obvious that the frequency and density of the weed species in the common barley fields will decrease losses of the common barley crop yield. Proper control of the weeds will increase the yield of common barley cultivation and total grain production. In this context, field observations to make necessary and collect information about the frequency and density of the weeds, to evaluate the economic losses caused by the weeds and to improve the new weed management tactics for the common barley production. In addition, it is concluded that weed needs to be investigated for the weed bank dynamics to a better estimate of the weed populations.

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Bayesian Network Analysis for the Factors Affecting the 305-day Milk Productivity of Holstein Friesians

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ABSTRACT
The variables affecting the milk productivity have been discussed in various articles through different methods. A recent study using path analysis shows that three variables significantly affect the 305-day milk yield of Holstein Friesian cows. These variables are parity, first calving year and lactation length. Calving season is another variable which appears to be significant in a different study. The aim of this study is to provide a simultaneous multilateral analysis among the milk yield, these three variables and a new variable calving season. The analysis was realized through a Bayesian network built over the findings of the path analysis. 17,109 records of Holstein Friesian cows calved between 2001-2011 years were analyzed. The estimated Bayesian network showed that younger cows produced more milk. Lactation length and parity do not depend on each other. Cows reached their highest amount of milk yield on their 4th parities. Milk yield is mostly affected by lactation length. Finally, first calving year, parity, lactation length and calving season should be considered as criteria in a selection study to increase the milk yield.

Keywords: 305-day milk yield; Holstein Friesians; Bayesian networks; Structure learning

1. Introduction

Yield is an important economic trait for dairy cattle. In order to reach a good level of productivity, factors affecting milk yield and identifying and analyzing their effects are important issues. In the literature, various studies about milk yield exist. Perochon et al (1996) have modeled the lactation curves of dairy cows with an emphasis on individual variability. They have found that the quality of the individual prediction was better for French Friesian and Montbeliarde cows than for pure or crossbred Holstein cows. İnci et al (2007) have investigated the milk yield and reproductive traits of Brown Swiss cattle raised at Altınova State Farm and they found that the milk yield is highly affected by lactation length and period. Aktürk et al (2010) have studied on the factors affecting milk yield and milk production cost and they have found that silage maize (20%) and barley (14%) have the biggest effect on the milk yield. Tahtalı et al (2011) performed a path analysis to examine the factors affecting milk productivity of Brown Swiss cows and they calculated the percentages of direct effects of factors on actual milk yield as 17.3% for lactation length and 1% for 305-day milk yield. A study performed by İşçi et al (2015) showed that milk yield was significantly affected by three variables: parity, first calving year and lactation length. They applied a path analysis to indicate the significance of those three variables affecting the 305-day milk yield of Holstein

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Friesian cows. They also provided information about some bilateral relations among these variables. Iqbal et al (2016) have proposed a manually constructed Bayesian network model which assumes that the body condition parameter, the number of days after calving, and parity number affect the estrous cycle of the cattle. Using this model, they analyzed the dairy cattle productivity. Mote et al (2016) examined the effect of different macroclimatic variables on lactation milk yield and lactation length of Holdeo (Holstein Friesian x Deoni) crossbred cattle. Their regression analysis showed that calving season significantly affects the lactation milk yield. Eetvelde et al (2017) studied the factors associated with first-lactation milk yield in Holstein Friesian heifers and they found that the season of birth, but not calving, had a significant influence on the milk yield. Verma et al (2017) reported that, cows reach their highest amount of milk on their 4th parities. Eastham et al (2018) examined the associations between age at first calving and subsequent lactation performance of UK Holstein Friesian cows. They reported that lower first calving age leads to more amount of milk yield. However, there is not a previous work in the literature concerning milk productivity through a Bayesian network model.

Bayesian statistics assumes that parameters are not constants but variables. Moreover, in this approach, along with the sample information (likelihood), any existing information or experience about the parameter to be estimated are taken into account in a distribution form which is briefly called prior information. After combining the prior information with the likelihood function obtained from the observations, the posterior distribution is achieved. Therefore, using a Bayesian approach in a milk yield estimation study, one can add the results obtained in previous studies into the analysis to improve the quality of the estimations. Hence, the aim of this article is to provide a detailed multilateral analysis of the relationships among milk yield, these three variables affecting milk yield and a new variable calving season as well as to introduce the use of the Bayesian network model in milk productivity.

2. Material and Methods

The material of this study consisted of the 305-day milk record of the Holstein Friesian cows that calved between 2001-2011 years. 17,109 records, obtained from 1840 herds belonging to the members of Cattle Breeders Association in Isparta province, Turkey, were analyzed by building an appropriate Bayesian network.

Bayesian networks are a kind of probabilistic graphical models based on the Bayes' formula. Using a Bayesian network model, all conditional dependencies can simultaneously be calculated for a set of variables. Moreover, any prior knowledge about the relations among the variables can be added into the analysis. Bayesian networks correspond to the Directed Acyclic Graph (DAG) structure. Hence, a Bayesian network model has two main parts. The first part is the graphical part including the nodes (variables) with non-overlapping levels and directed arrows (edges) among the nodes. The second part consists of the conditional probabilities (parameters) usually displayed in a table called Conditional Probability Table (CPT). A CPT of a node gives the probability distribution of the variable represented by that node, conditional on its parent node. A directed arrow from one node to another indicates dependence among the variables represented by those two nodes. Any node from which an arrow comes out is called the parent node and the node that the arrow goes in is called the child node. All nodes following a path formed by the direction of certain arrows are called relative nodes. Among the relative nodes, the nodes on the path behind a certain node are called ancestors, and the ones afterwards are called descendant nodes. In a DAG, it is impossible to return to the same node following the path of an arrow directed from that node which means it is impossible for a node to be its own ancestor or descendant. Nodes are independent of their non-descendant nodes, given the state of their parents. An example of a Bayesian network model is presented in Figure 1. The Bayesian network shown in Figure 1 has five nodes named C1, C2, C3, C4 and C5. The conditional probabilistic relations among them are represented by the directed arrows. For example, C1 is a parent node of C2, while C2 is a child node of C4. It is also seen that, in accordance with the nature of the DAG structure, it is not possible to return to the same node following the path of an arrow directed from that node.

Let $X = (X_1, X_2, \dots, X_n)$ be the set of variables of a Bayesian network. Then, a Bayesian network simply represents a joint probability function such as

$$P_B(X_1, X_2, \dots, X_n) = \prod_{i=1}^{n} P_B(X_i \backslash \pi_i) \quad (1)$$

Where; X_i are the nodes representing variables and π_i is any conditioned state of a node or an evidence. Evidence can also be defined as an observed or occurred value of a variable. Inference in Bayesian networks is made by the propagation of the evidences through the network. For any evidence, π_i the joint posterior probabilities $P_B(X_1, X_2, \dots, X_n)$ in Equation 1 can be obtained.

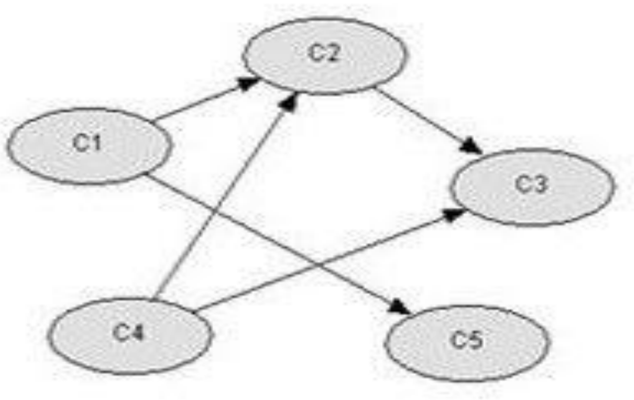

Figure 1- Example of Bayesian network

Building a Bayesian network takes two main tasks: construction of the graphical structure of the network and calculation of the posterior probabilities. There are three main approaches to building the graphical structure of Bayesian networks. In the first case, dependencies among the variables, that is, directions of the arrows are determined by a specialist of the subject manually. In the second approach, however, the structure of the network is learned directly from the data set through some learning algorithms. This type of building a Bayesian network is called structure learning. The third approach called the hybrid approach is a combination of the first two ones. In this type of building a Bayesian network, a specialist constructs the graphical structure of the network manually then an algorithm is used to learn the parameters or the specialist provides background information to an algorithm. For more detailed information about the construction of Bayesian networks, see Jensen (2001).

In structure learning, there are various algorithms for building a Bayesian network. These algorithms can mainly be classified as constraint-based and score-based algorithms. Constraint-based algorithms are based on an algorithm called Inductive Causation algorithm by Verma & Pearl (1991). This type of the algorithms decides for the existence and direction of an edge through some conditional independence tests. More detailed information about constraint-based algorithms exists in Spirtes et al (1993), Cheng et al (2002), de Campos & Huete (2000). Score-based algorithms choose the graph structure having the highest score, after calculating a score value for all possible candidate Bayesian network structures. Detailed information about score-based algorithms can be found in Cooper & Herskovits (1991), Heckerman (1998), Chickering (2002).

In general, constraint-based algorithms are less time consuming and more powerful algorithms compared to the score-based algorithms (Natori et al 2015). There are various computer packages that can be used to build a Bayesian network such as GeNIe (2019), Hugin (2019), Netica (2019). To measure the estimation performance and uncertainty of Bayesian networks, there are various metrics. Marcot (2012) provides detailed information about those metrics and their applications. Among these metrics, the logscore value can be used as a performance evaluation measure for a Bayesian network and it is calculated as follows.

$$logscore(X_1, \dots, X_n) = \frac{\sum_{i=1}^{n} log_2 P(X_i/model)}{nN} \quad (2)$$

Where; n is the number of cases in the test set and N is the total number of nodes in the model. The highest the logscore is, the better is the model. A sensitivity analysis for a Bayesian network model can determine how much the nodes are affected by the changes in the other nodes on the network. Model sensitivity can be measured by entropy scores. Bayesian estimation approach takes into account the prior information unlike the classic approach.

In this research, the data consist of the values belonging to four variables: 1) first calving year, 2) lactation length, 3) parity and 4) calving season. The variable first calving year denotes the life year (first year, second year etc.) or simply the age of the animal in which the cow gave birth to its first calf. First calving year is an important variable showing the fertility rate of a cow. Because we need the non-overlapping nodes of these variables as the levels of the network, we classified each variable using appropriate interval in accordance with dairy science.

Furthermore, prior information from a former study about the relations among those variables is available such that, İşçi et al (2015) have shown that milk yield is significantly affected by three variables: first calving year, lactation length and parity. Similarly, Mote et al (2016) stated that calving season is also a significant variable affecting the milk yield. Therefore, in accordance with the nature of the Bayesian estimation, it is appropriate to adopt a hybrid approach to build the network. Hence, at the stage of deciding for an appropriate algorithm, with the help of GeNIe (2019) software, Bayesian Search algorithm provided by Cooper & Herskovits (1991), PC algorithm (named after its authors, Peter and Clark) given by Spirtes et al (1993) and Greedy Thick Thinning algorithm given by Cheng et al (2002) have been employed along with the prior information providing a constraint to be imposed on the initial directions of the edges among the nodes to be forming the network. Also, the entropy scores were calculated by Netica (2019) software. The main differences between these algorithms are: Bayesian Search is a score-based algorithm while PC algorithm is a constraint-based algorithm ordering the conditional independence tests from small to large which makes it an efficient algorithm. Greedy Thick Thinning algorithm is also a score-based algorithm based on the Bayesian Search algorithm. There are some differences among their mechanisms such as Bayesian Search algorithm produces a DAG that gives the maximum score which is proportional to the probability of the structure given by the data while PC algorithm uses the independences detected in data through some independence tests to estimate the structure of the network. Greedy Thick Thinning algorithm, however, starts with an empty graph and repeatedly adds the arcs until no arc addition causes a positive increase.

3. Results and Discussion

At the stage of building the Bayesian network, three different Bayesian networks based on the PC, Greedy Thick Thinning and Bayesian Search algorithms were developed. Afterwards, their estimation performances based on the logscore values given by the Equation 2 were calculated. Table 1 shows the model prediction performances of those algorithms in logscore values. The algorithm with a higher logscore builds a model that has a better estimation performance. In Table 1, it is seen that PC algorithm has the biggest logscore of -676.90. Hence, it can be concluded that the model estimated by the PC algorithm fits the data the best. The second best performing model was estimated by the Greedy Thick Thinning algorithm with a logscore of -678.04 and the third was the model estimated by the Bayesian Search algorithm with a logscore of -679.09. Therefore, the Bayesian network estimated based on the PC algorithm was adopted, visualized and given in Figure 2. The estimated Bayesian network displays the conditional relationships and the initial probabilities belonging to the states of the nodes first calving year, lactation length, parity, calving season and milk yield. Propagating any evidence into the network, the posterior probabilities of all the nodes can be calculated. Sensitivity analysis measures how much a node in a Bayesian network is affected by the changes in the levels of other nodes. That measurement can be made by the entropy reduction scores given by Pearl (1991).

Table 1- Candidate algorithms and their logscore values

Algorithm	*Logscore*
PC	-676.90
Greedy thick thinning	-678.04
Bayesian Search	-679.09

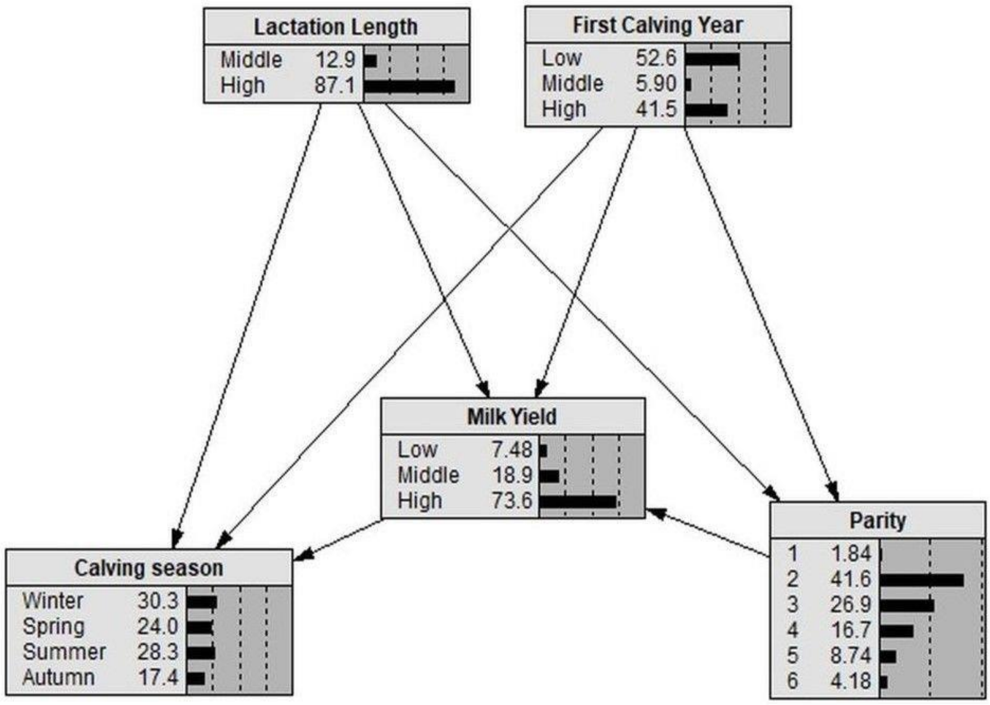

Figure 2- Bayesian network model for the factors affecting the milk productivity of Holstein Friesian cows

$$I = H(Q) - E(Q \backslash F) = SUM \sim qSUM \sim fP(q,f)log(P(q,f)/[P(q)P(f)]) \quad (3)$$

In Equation 3, Q is the query variable, F is the varying variable, q is a state of the query variable and f is a state of the varying variable. $H(Q)$ is the entropy of Q before any new findings, $E(Q \backslash F)$ means the expected real value of Q after new finding f for node F. $SUM \sim q$ means the sum over all states q of Q. Hence, to see how much milk yield is affected by the changes in the levels of other nodes i.e. lactation length, calving season, parity and first calving year, the calculated entropy scores for each node are given in Table 2. As seen from the entropy scores provided in Table 2, milk yield is mostly affected by the lactation length (0.12126). Afterwards, the second most effective variable is calving season (0.08136), the third is the parity (0.06567) and the last is the first calving year (0.00091). After estimating the Bayesian network for the factors affecting the milk productivity of Holstein Friesian cows, it is possible to perform a simultaneous analysis among those factors using this network. That is, it is possible to observe how the levels of the other variables will change simultaneously, when an evidence for any node is propagated through the network. Below are some remarkable results of the analysis.

Table 2- Entropy scores of the variables affecting the milk yield

Variable	*Entropy score*
Lactation length	0.12126
Calving season	0.08136
Parity	0.06567
First calving year	0.00091

- The highest amount of milk yield is obtained from the cows having low first calving years. The second highest amount of milk is produced by the cows having high first calving years. At middle first calving ages, cows are more likely to produce middle amount of milk. Hence, heifers should be bred at young ages. This result is in accordance with the results given by Kaya et al (1998) who found that breeding the heifers at young ages will increase the milk yield as well as lowering the cost of raising. Eastham et al (2018) also stated that lower first calving ages leads to more amount of milk yield. This result is also supported by Şekerden & Özkütük (2000) pointing out that for the productivity of a cow per day to be maximum, its first breeding age must be low. First breeding can be done when the cows reach 67% of their adult age (Tümer 2001). Holstein Friesian heifers raised in Turkey can be bred between 14th and 15th months with high level care and feeding (Yüksel et al 2000).

- Cows reach their highest amount of milk yield on their 4th parities. This result is in accordance with the result given by Verma et al (2017). Cows on the first and the sixth parity tend to produce less amount of milk than other periods.

- Cows having middle lactation length tend to produce the middle amount of milk, while the ones having high lactation length produce a high amount of milk. Cows calving in spring and summer seasons tend to have the middle lactation lengths. However, the ones calving in winter and autumn seasons mostly have high lactation length. Therefore, this result complies with the other finding showing that the most amount of milk is obtained in winter and autumn seasons compared to the other seasons. These results are also supported by the findings given by Mote et al (2016) who observed that the average lactation milk yield and lactation length is more in winter season. However, Eetvelde et al (2017) stated that season of calving did not actually influence the milk yield and the seasonal differences were due to the psychological features caused by the seasonal metabolic adaptations of the animals.

- Cows having high lactation lengths calf in winter at the highest rate while the ones having middle lactation lengths mostly calf in spring. This result is also in accordance with the previous comment that points out the highest milk yield is obtained in winter with the highest rate.

- Cows calving for the first time in low ages seem to calf in winter with the highest rate. However, the ones calving in middle and high ages mostly seem to calf in summer.

- Cows having low first calving year and high first calving year tend to have more high level of milk yield than the ones having middle first calving year.

- According to the sensitivity analysis, milk yield is mostly affected by lactation length, secondly it is affected by calving season and thirdly by parity and finally by the first calving year. This result slightly differs from the results that are given by İşçi et al (2015) who have found that the effect of the lactation length on milk yield is of second importance. This difference can be explained by the difference in the herd management factors as location, climate and diets of the animals under consideration. Furthermore, İşçi et al (2015) remarks that in a selection study to increase the milk yield, first calving year and lactation length should be considered as selection criteria. However, we suggest that calving season and parity should also be included in those criteria.

4. Conclusions

In conclusion, using a Bayesian network has some advantages compared to the other analysis methods. Unlike the other methods, previous findings of the variables and their known relations can be added into the Bayesian model under the name "prior information". In this study, the estimated Bayesian network was built on two different prior information. The first one is that the milk yield was significantly affected by three variables: parity, first calving year and lactation length and secondly calving season is another factor affecting milk yield significantly. The prior information was inserted into the network by directing arrows manually from those four nodes into the milk yield node. Afterwards, when the real structure of the Bayesian network was obtained based on the current data set, the prior relations among the variables were updated. This feature of the Bayesian networks allows current data to update the findings of the old information. After this updating process, new findings can also be used as prior information for another study. Hence, Bayesian networks have the property of recycling the information. Therefore, instead of ignoring or excluding the previous information, Bayesian networks include them in the analysis. Another advantage of the Bayesian networks are they do not need to assume any probabilistic distribution. They can be applied to continuous, discrete or categorical data. Moreover, as well as observing the effects of the variables on the target variable (the milk yield in this study), one can also observe how variables affect each other as well. In this research, the data consist of the structure of the Bayesian network observing the relations among the variables is easy and can be made simultaneously. When the corresponding levels of the desired nodes are selected on the network (propagation process), the response in the other nodes conditional to the selected values can immediately be observed. Basically, this can be though as a very fast conditional probability calculation based on complex conditional probabilistic relations represented by the Bayesian network estimated.

The theoretical and practical advantages of Bayesian networks given above can be successfully adapted in milk productivity or any other farming area. In fact, Bayesian networks are quite in accordance with the natural mechanism of milk production. There are not just direct relations between the milk yield and the factors affecting it. Instead, there are also complex relations among the variables affecting the milk yield. Hence, in farm management, to increase the milk productivity, while changing a factor that is known to be effective on milk yield, one must also consider its indirect effects due to the changes in other factors caused by the factor under consideration. For example, cows calving in spring and summer seasons mostly seem to have middle lactation length, and as the Bayesian network indicates middle lactation length is a factor causing middle amount of milk. Hence, it is obviously seen that calving season has both direct and indirect effects on the milk yield.

Examining the factors affecting milk productivity and the relations among them will increase the milk productivity as well as the success of future improvement studies. The study showed that in a selection study choosing the cows having high lactation lengths and parity for breeding or adjusting the calving season and first calving year time will be useful. These improvements will increase the genetic quality of the cows and the milk productivity.

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The Effect of Glyphosate on Anatomical and Physiological Features of Alfalfa Infested with Field Dodder (*Cuscuta campestris* Yunck.)

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ABSTRACT
Field dodder (*Cuscuta campestris* Yunck.) is a very harmful parasitic weed species worldwide which infests many crops, including alfalfa as a foremost forage crop. Glyphosate has been an effective herbicide for field dodder control even though side effects occasionally occur in alfalfa plants. To find out and quantify the effects of glyphosate (288 and 360 g a.i. ha^{-1}) on field dodder control, alfalfa forage yield, and physiological and anatomical features of alfalfa plants under controlled conditions were aims of the study. Physiologic (chlorophyll a, chlorophyll b, total carotenoids); and anatomic parameters were measured. Leaf anatomic parameters were thicknesses of upper epidermis, palisade and spongy tissues, mesophyll and underside leaf epidermis, and diameter of bundle sheath cells. Stem anatomic parameters were thicknesses of epidermis and cortex, and diameters of stem and central cylinder (pith). Both rates of glyphosate caused recovery of the harmful effects of field dodder on alfalfa, which shows that glyphosate can control field dodder at early stages of infestation on alfalfa.

Keywords: Glyphosate; Field dodder; Leaf parameters; Pigments content; Stem parameters

1. Introduction

Alfalfa is an important forage crop in Serbia, covering approximately 200 000 ha, as well as worldwide (Bokan et al 2015; Strbanovic et al 2017). *Cuscuta* spp. are present in anthropogenic habitats in Serbia from croplands to urban areas and have been recorded at 25% of the UTM (Universal Transverse Mercator) grid, becoming a foremost problem in alfalfa cultivation in Serbia (Vrbnicanin et al 2008). Field dodder (*Cuscuta campestris*) is the most common *Cuscuta* species globally as it has been recorded on many crop and non-crop species in South America, Europe, Asia, Africa, and Australia, as well as its native range North America (Holm et al 1997; Saric-Krsmanovic et al 2015). Alfalfa losses due to field dodder have been reported to reach up to 80% (Stojanovic & Mijatovic 1973; Dawson 1989; Mishra 2009). Its damaging effect is more pronounced in newly established alfalfa fields (Dawson 1971).

Field dodder management requires an integrated approach, which targets from the introduction of parasite seeds to the field to avoid further dispersal of seeds by using different techniques such as preventive, cultural, biological, mechanical and chemical (Saric & Vrbnicanin 2015). Chemical control of field dodder is a method that has been

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predominantly studied worldwide (Dawson 1966; Saric & Vrbnicanin 2015; Boydston & Anderson 2017). One of the herbicides that have been studied and suggested for field dodder control in alfalfa is glyphosate, phloem and xylem mobile systemic herbicide, which reaches areas of active growth through symplastic and apoplastic pathways (McAllister & Haderlie 1985). Liu & Fer (1990) found out that foliar applied glyphosate accumulated 26-fold more apical parts of dodder plants than hosts (Hock et al 2008). Glyphosate primarily inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway. Glyphosate sprayed to dodder parasitized alfalfa at different rates caused injury to death of the field dodder while there was no injury to minimal damage on alfalfa in various studies (Dawson & Saghir 1983; Dawson 1990a; Dawson 1990b; Saric-Krsmanovic et al 2015). But, some regeneration of field dodder occurred in some cases and alfalfa plants recovered from injury in most cases (Dawson & Saghir 1983; Dawson 1990a). In pot and field trials, glyphosate has demonstrated better results than other herbicides; however, the effectiveness of the herbicides, including glyphosate, was lower in heavily infested alfalfa fields (Saric-Krsmanovic et al 2015).

The effect of field dodder on physiologic and anatomic parameters of crops and their relation with herbicides have been reported. Field dodder reduced the contents of carotenoids, chlorophyll a, and chlorophyll b both sugar beet and alfalfa plants but herbicide treatments (imazethapyr in alfalfa and propyzamide in sugar beet) caused partial recovery of all three parameters, even with small level of stimulations 28 and/or 35 days after herbicide applications (Saric-Krsmanovic et al 2016; Saric-Krsmanovic et al 2017). Field dodder significantly decreased all measured anatomic features of leaf and petiole of sugar beet plants, compared to noninfested ones; but, propyzamide treatment caused different levels of recovery of all parameters, inferring that propyzamide could control field dodder in early infestation stages (Saric-Krsmanovic et al 2017). Anatomic parameters of alfalfa stem and leaf were also studied under field dodder infestation and imazethapyr treatment. Field dodder caused some reductions in the parameters, but they were significant from the 28th day onwards. Imazethapyr at 100 g a.i. ha^{-1} application rate resulted in a partial recovery of the measured parameters (Saric-Krsmanovic et al 2016).

Field dodder has been considered a growing problem in alfalfa production in Serbia and other countries, which indicates a need for more extensive research aimed at finding new means of parasitic weed control. Glyphosate has been confirmed as a viable option for dodder control in alfalfa crops. The effects of field dodder infestation of alfalfa have been reported (Saric-Krsmanovic et al 2016).The aim of the present study is to find out the effects of glyphosate application on physiologic and anatomic parameters and yield of alfalfa.

2. Material and Methods

Pot experiments were set up in a greenhouse with 28±3 °C ambient air temperature and under natural light conditions in June 2015. Physiologic and anatomic measurements were done in the laboratory. In general, the same methods were applied as those reported by Saric-Krsmanovic et al (2016; 2019).

2.1. Greenhouse experiment

Plastic pots (17 cm in diameter) were filled with a mixture of a commercial substrate (Flora Gard TKS1, Germany) and soil that collected from a field without a history of herbicide application, which was loamy in texture (sand 49.8%, silt 33.4%, and clay 16.8%), medium calcareous, weakly alkaline and highly humic. Twenty plants remained in each pot after thinning. Glyphosate was applied at 288 g a.i. ha^{-1} (T_1) or 360 g a.i. ha^{-1} (T_2) rates by a thin-layer chromatography sprayer under 1-2 bars pressure over alfalfa plants at 10-12 cm in height and field dodder have already attached. Two different checks were included in the experiment: alfalfa plants noninfested with field dodder (N) and infested with field dodder (I), but neither treated with glyphosate. The experimental design was an RCBD with four replications; the experiment was repeated twice. Herbicide efficacy was assessed visually assessing field dodder plants on alfalfa by using a 0 (no damage) -100 (fully death) scale and by weighing fresh biomass of alfalfa at 0, 7, 14, 21, 28, 35 days after application (DAA).

2.2. Physiological analysis - content of pigments

The content of pigments was measured spectrophotometrically following to methanol extraction 0, 7, 14, 21, 28 and 35 DAA. Leaves (0.5 g), which had been kept in -20 °C until the processing were ground in a blender with 5 mL methanol followed by vacuum filtering and centrifuging for 10 min at 1500 rpm. Absorption was read at 470 nm for total carotenoids, at 666 nm for chl *a* and 653 nm for chl *b*. Wellburn's formula (1994) to calculate chlorophyll *a* and *b* concentration, and Lichtenthaler & Wellburn's formula (1983) for carotenoids were used.

Chlorophyll *a*: ca= 15.65xA666-7.34A653 (1)

Chlorophyll *b*: cb= 27.05xA653-11.21A666 (2)

Total carotenoids: cx+c= (1000A470-2.86 ca-129.2 cb)/221 (3)

Pigment content was converted from µg mL^{-1} to mg g^{-1} using the Equation 4.

$C = cVR\ (m1000)^{-1}$ (4)

Where; C, pigment content (mg g^{-1}); c, pigment content (µg mL^{-1}); V, total extract volume (mL); R, dilution factor; m, fresh weight of leaves (g).

2.3. Anatomical analysis

Leaves and stems were sampled for light microscopy six times: 0, 7, 14, 21, 28 and 35 DAA. Samples were obtained by cutting 1-cm-long alfalfa stem parts where field dodder contacted alfalfa and collecting 10 of the first three true leaves from each level of plants, namely the lower, middle and upper. The samples were fixed in 50% ethanol solution and prepared for microscopy using paraffin wax method (Ruzin 1999). Embedded samples were then sliced into 5-15 µm thick sections on a microtome, then stained with toluidine blue, safranin and alcian blue. Stem parameters that were thickness of epidermis (TE), thickness of cortex (TC), diameter of stem (DS) and diameter of central cylinder (pith) (DCC); and leaf parameters that were thickness of upper epidermis (TUPE) and underside leaf epidermis (TUNE), thickness of palisade (TPT), spongy (TST) and mesophyll (TMT) tissues, and diameter of vascular bundle cells (DVBC) were measured as 30 replicates each. Measuring process completed using the LEICA IM 1000 software package followed to visualization by a light microscope LEICA DMLS and photographing by a digital camera LEICA DC 300.

2.4. Statistical analysis

The data were combined and underwent variance analysis and tested by Fisher's Least Significant Difference (LSD) test ($P<0.05$) by using Statistica 8.0. software. Principal component analysis (PCA) was run based on combinations of four treatments by six assessment times and four parameters of stem or six parameters of leaf anatomy of alfalfa in order to define the best control treatment with precision.

3. Results and Discussion

3.1. Effects of field dodder and glyphosate applications on alfalfa yield

The impact of field dodder on alfalfa fresh weight was significant. Noninfested alfalfa plants were gaining 18.35 g fresh weight per pot (from 8.00 g 0 DAA to 26.35 g 35 DAA) over the 35 days period, while field dodder-infested plants lost 2.70 g (measuring from 6.88 g 0 DAA to 4.18 g 35 DAA) (Figure 1). It is an 84% fresh fodder loss, which is unacceptable to farmers. Alfalfa crop losses due to field dodder had been assessed in earlier studies at 60% in Chile, and 80% in Serbia (Stojanovic & Mijatovic 1973; Mishra 2009). In a three-year field trial alfalfa loss due to smoothseed dodder (*Cuscuta approximata*) compared to uninfested alfalfa plots changed from 7% to 70% in different years (Tepe et al 2017). The growth of field dodder was faster than that of *Mikania micrantha*, an invasive alien plant in China, and damage caused by field dodder qualified it a biological agent to control *M. micrantha* (Deng et al 2003; Zan et al 2003; Zhang et al 2004; Li et al 2012). Similarly, field dodder was found promising in control of *Ambrosia trifida*, an invasive alien plant in Europe (Vrbnicanin et al 2013).

The efficacy of both rates of glyphosate was significant compared to infested herbicide-untreated alfalfa plants. Notably, the higher rate (T_2) gave the same yield as noninfested alfalfa plants (Figure 1). Visual estimation of field dodder damage was in parallel to fresh weight data, which was over 90% 28 DAA and beyond (Table 1). These results are consistent with earlier studies for field dodder control in various crops (Dawson 1990a, b; Hock et al 2008; Saric-Krsmanovic et al 2015).

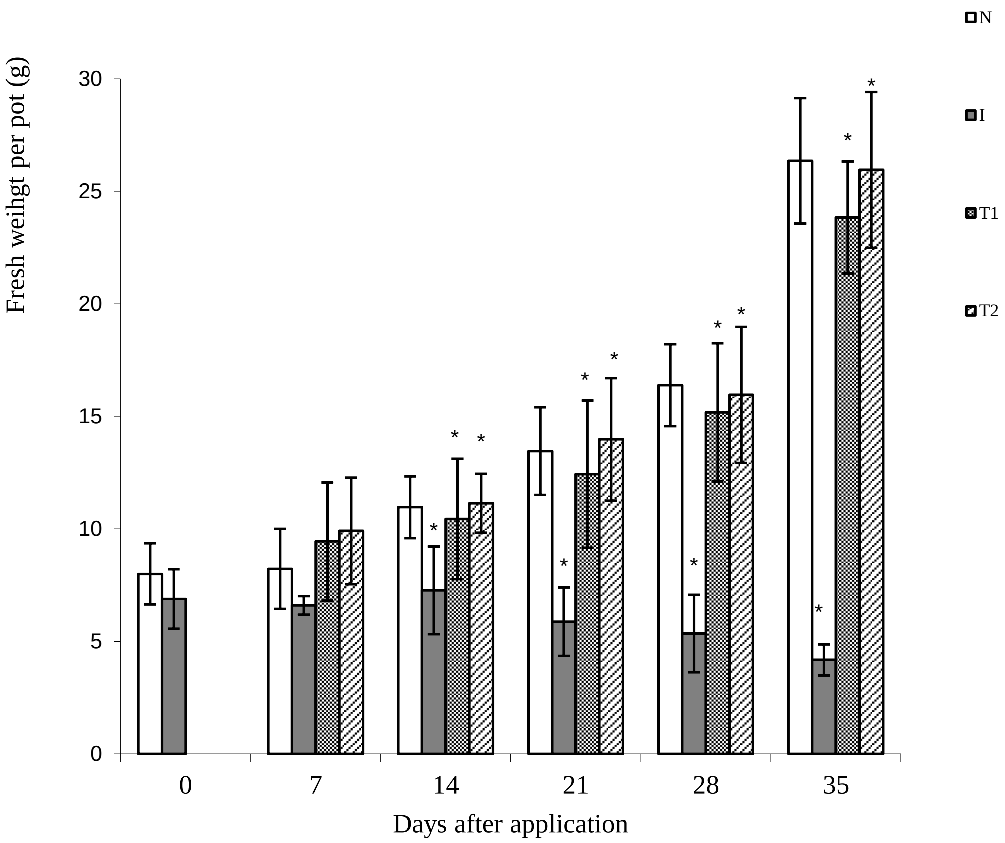

Figure 1- Effects of field dodder infestation and glyphosate treatments on alfalfa. N, noninfested alfalfa plants; I, infested with field dodder but untreated with glyphosate; T_1 and T_2, treated with glyphosate (288 and 360 g a.i. ha^{-1}); * LSD test, P<0.05

Table 1- Visual assessment of glyphosate efficacy on field dodder plants parasitizing alfalfa

Treatments (g a.i. ha^{-1})	*Assessments in the days after application (%)*				
	7	*14*	*21*	*28*	*35*
Glyphosate 288	3.0	70.0	87.5	93.0	95.0
Glyphosate 360	5.0	75.0	90.0	97.5	97.5

3.2. The effect of glyphosate application on physiological parameters of alfalfa

The results of measurement of physiological parameters are presented in Figure 2. As discussed in an earlier paper, untreated alfalfa plants had reduced contents of chlorophyll *a* up to 64%, chlorophyll *b* 68%, and carotenoids 60% on the 35th DAA (Saric-Krsmanovic et al 2016). Two glyphosate treatments gave very similar results, showing that herbicide-treated alfalfa plants had similar amounts of all three pigments as noninfested alfalfa plants, especially 28 DAA and beyond. Even a small amount of stimulation of carotenoids content by T_2 application was measured (Figure 2). The effect of glyphosate application on dodder-infested alfalfa is consistent with the effect of imazethapyr on alfalfa and propyzamid on sugar beet (Saric-Krsmanovic et al 2016; Saric-Krsmanovic et al 2017).

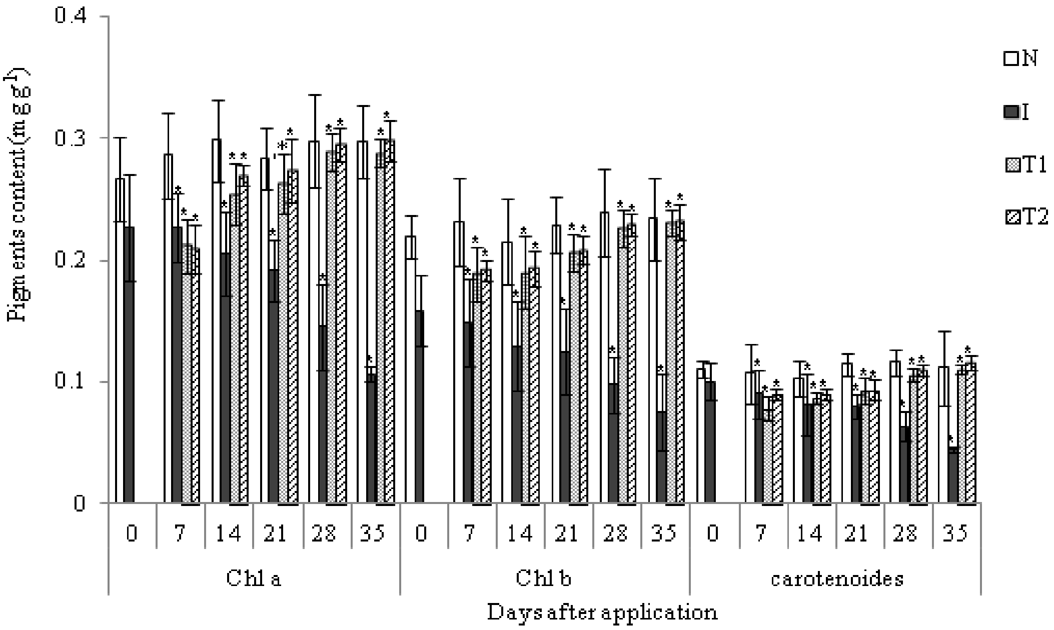

Figure 2- Contents of chlorophyll *a* (a), chlorophyll *b* (b) and carotenoids (c) in alfalfa plants. N, noninfested alfalfa; I, untreated with herbicide; T_1 and T_2, glyphosate-treated (288 g a.i. ha^{-1} and 360 g a.i. ha^{-1}); * LSD test, $P<0.05$

3.3. The effect of glyphosate application on anatomical parameters of alfalfa

Data for all physiologic parameters of plants, including noninfested and untreated plants, are presented here to be able to make a meaningful explanation, although results other than glyphosate data had been reported earlier, which details can be reached at Saric-Krsmanovic et al (2016).

Leaf heights data were pooled because the leaf position did not significantly affect the parameters measured. Both glyphosate rates affected all leaf parameters to very similar degrees in an increasing manner in time. Field dodder caused reductions in the parameters, but they were significant 28 DAA and afterwards (Table 2). The highest reduction was revealed for mesophyll thickness (57%), and the lowest for the thickness of upper epidermis (28%). Neither rate caused a full recovery of any parameter; however, they almost reached the level of noninfested plants 35 DAA. The results were consistent with earlier studies with different herbicides applied to alfalfa and sugar beet plants (Saric-Krsmanovic et al 2016; Saric-Krsmanovic et al 2017). The PCA analysis showed that total variability among all parameters over all assessments was 78% (PCA1 axis) and 11% among treatments (PCA2 axis) (Figure 3). Treatments have had the most significant impact on TMT, TPT, and TUNE; but, their influences on DVBC, TST, and TUPE were smaller. Treatments T_1 and T_2 in the lower left-hand quadrant of the ordination plane had higher impact on TUNE, TUPE, and TPT than on the other parameters. The least difference was found in the leaf anatomy parameters of control alfalfa plants and those in treatments T_1 and T_2.

As leaf parameters, stem parameters also showed lower reductions in treated plants than in dodder-infested untreated plants, ranging from 15% to 0% (Table 3). Recovery from both glyphosate rates was very similar, and pronounced on the 28th DAA and beyond. PCA1 axis confirmed a variability of 80%, including all measured parameters over all assessments. Treatment variability of 13% was confirmed by PCA2 axis (Figure 4). Treatments had the highest impact on TE, DCC, and DS and lower effect on TC. Treatments T_1 and T_2 (shown in the lower left-hand quadrant of ordination plane) had the highest impact on TC and TE. Besides, the two last assessments after herbicide treatments (T_1 and T_2) confirmed their significant impact on field dodder plants, i.e., the parameters of alfalfa stem anatomy in those treatments and the control had the least difference (Figure 4). Data showed a recovery of the infested alfalfa plants and satisfactory efficacy of the herbicide.

Table 2- The effect of glyphosate treatments on alfalfa leaf parameters

Parameter (µm)	*App*	*Days after application*					
		0	*7*	*14*	*21*	*28*	*35*
Thickness of upper epidermis	N	15.5±5.3	15.6±2.8	17.0±3.8	17.7±4.6	19.0±3.5 a	19.2±2.8 a
	I	14.4±4.6	15.3±3.7	14.2±4.1	14.1±2.4	14.1±2.7 b	13.8±4.3 b
	T_1	-	15.8±2.6	15.3±4.3	15.0±2.5	16.2±4.2 c	17.2±3.5 ac
	T_2	-	15.1±3.5	15.5±2.4	15.2±2.9	16.0±3.6 c	18.3±4.9 ac
Thickness of palisade tissue	N	45.5±7.9	54.7±13.7	54.8±9.2	54.9±15.5	55.0±21.8 a	66.7±14.15 a
	I	45.2±10.1	44.5±9.8	44.1±7.1	43.0±7.9	42.7±9.3 b	35.0±10.8 b
	T_1	-	41.0±11.8	44.9±8.7	51.5±14.5	55.7±10.3 ac	64.8±8.4 ac
	T_2	-	41.0±9.2	47.0±12.0	48.4±15.0	50.2±10.7 c	62.8±8.7 ac
Thickness of spongy tissue	N	47.2±10.6	50.1±15.6	52.5±8.9	52.8±16.9	54.5±11.2 a	66.6±15.0 a
	I	46.5±10.8	45.7±10.7	44.7±10.3	43.0±8.0	41.4±16.9b	32.8±9.0 b
	T_1	-	39.4±10.5	47.2±14.1	48.9±9.2	49.7±8.4 c	65.4±9.9 ac
	T_2	-	42.2±7.8	45.9±11.4	49.0±10.9	50.0±10.9 c	63.6±27.0 ac
Thickness of mesophyll tissue	N	14.0±4.4	14.1±3.7	14.8±3.4	15.3±4.1	15.7±3.6	16.8±6.2 a
	I	13.6±3.8	13.5±3.1	13.5±4.8	13.2±4.3	13.2±3.5	7.2±3.6 b
	T_1	-	13.6±2.4	13.3±3.1	14.1±4.6	14.7±3.2	16.6±5.4 ac
	T_2	-	12.8±2.5	13.5±3.7	15.1±4.4	15.4±4.3	15.8±5.0 ac
Thickness of underside epidermis	N	93.5±22.5	106.1±28.4	106.8±18.8	105.5±34.4	111.0±22.6 a	112.1±28.8 a
	I	91.5±15.6	90.6±17.4	88.8±13.9	87.6±14.6	85.8±24.8b	69.2±21.1b
	T_1	-	80.2±20.9	91.1±15.5	94.3±26.0	109.0±21.6 ac	111.4±14.7 ac
	T_2	-	83.3±18.9	93.0±21.0	100.1±22.4	102.2±16.0 c	110.9±32.6 ac
Diameter of vascular bundles cells	N	12.3±4.8	12.7±2.8	13.1±4.3	13.3±2.6	13.6±4.6 a	13.9±4.1 a
	I	11.6±3.1	11.8±3.3	10.1±2.8	10.0±2.7	9.3±2.7b	9.9±2.2 b
	T_1	-	9.8±1.1	9.9±2.8	13.1±3.9	14.3±3.4 ac	13.4±3.3 ac
	T_2	-	9.6±2.1	10.9±3.4	12.8±3.1	13.2±2.8 ac	13.2±3.3 ac

Means are followed by the relative standard errors and and letters which mean that the same letters within a column are not significantly different according to LSD test ($P < 0.05$).

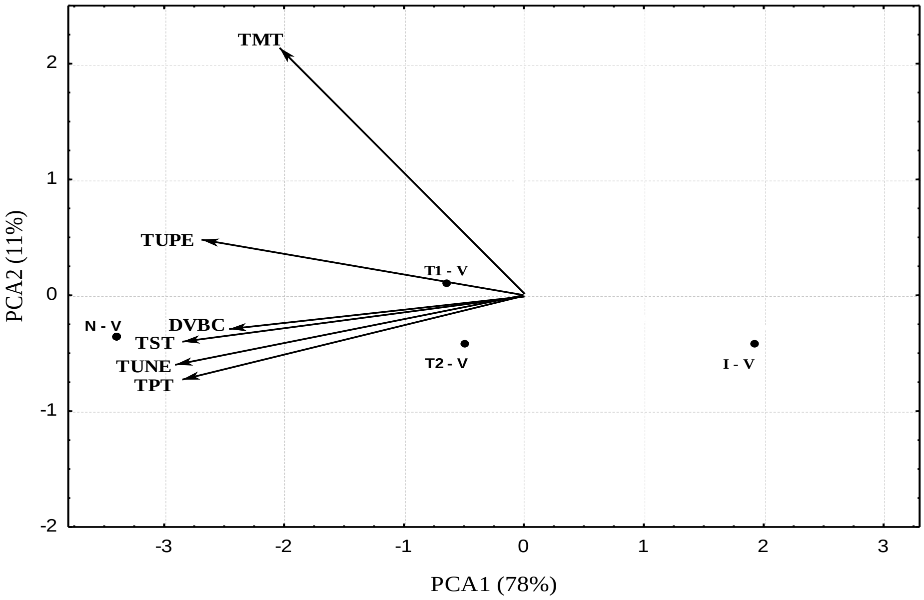

Figure 3- Principal component analysis for leaf parameters of alfalfa plants in the last assessment (35 DAA). N, noninfested by field dodder, T1 treated with glyphosate (288 g a.i. ha-1); T2 treated with glyphosate (360 g a.i. ha-1); I, untreated; TUPE, thickness of upper epidermis; TUNE, thickness of underside leaf epidermis; TPT, thickness of palisade tissue; TST, thickness of spongy tissue; TMT, thickness of mesophyll tissue; DVBC, diameter of vascular bundle cells

Table 3- The effect of glyphosate treatments on alfalfa stem parameters

Parameter (µm)	*App*	*Days after application*					
		0	*7*	*14*	*21*	*28*	*35*
Thickness of epidermis	N	16.1±4.0	17.5±5.3	17.6±2.9	17.9±5.8	18.7±4.2 a	20.2±4.2a
	I	15.5±3.8	14.4±4.2	14.3±2.8	13.9±2.2	13.5±3.1 b	13.2±4.1b
	T_1	-	10.9±4.0	13.4±2.0	15.1±4.4	16.0±3.4 ac	17.9±6.1 ac
	T_2	-	15.5±3.2	15.5±2.4	16.2±2.6	16.5±3.3 ac	18.8±3.9 ac
Thickness of cortex	N	62.6±18.8	66.0±9.2	70.5±19.7	74.4±24.2	73.9±13.8 a	81.9±15.3 a
	I	59.1±14.7	55.2±13.7	53.0±15.3	52.3±12.6	52.0±12.2 b	39.9±9.4 b
	T_1	-	47.9±16.3	48.7±13.2	54.8±20.8	60.7±7.5 c	69.8±12.3 c
	T_2	-	49.5±8.3	59.3±17.1	61.0±8.6	63.7±21.0 c	77.4±18.4 c
Diameter of central cylinder	N	947.9±83.4	1043.3±237.9	1074.4±312.0	1138.6±209.8	1213.7±191.8 a	1227.5±244.3 a
	I	1017.2±182.4	977.9±172.2	952.3±166.3	950.4±90.9	948.1±81.0 b	814.9±92.7 b
	T_1	-	860.9±179.4	1142.4±225.5	1086.1±245.3	1197.1±160.6 ac	1225.0±264.7 ac
	T_2	-	897.0±164.3	917.4±226.0	1098.7±107.5	1157.8±169.6 ac	1227.6±127.5 ac
Diameter of stem	N	1154.1±104.8	1252.5±353.3	1240.2±236.4	1285.8±209.9	1343.1±195.7 a	1414.6±261.6 a
	I	1211.4±254.6	1159.2±150.2	1121.2±110.9	1085.7±171.9	1040.3±72.4 b	911.6±114.5 b
	T_1	-	937.5±195.5	1263.5±224.1	1347.6±304.8	1388.4±275.3 ac	1410.0±209.3 ac
	T_2	-	998.2±179.6	1011.7±166.2	1314.8±212.5	1317.7±109.3 ac	1413.8±180.7 ac

The same letters within a column are not significantly different according to LSD test ($P < 0.05$).

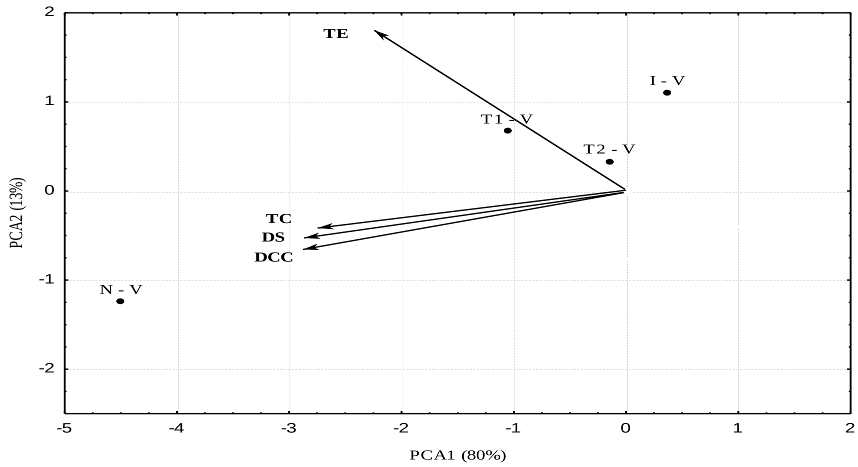

Figure 4- Principal component analysis for stem parameters of alfalfa plants in the last assessment (35 DAA). N, noninfested by field dodder, T_1 treated with glyphosate (288 g a.i. ha^{-1}); T2 treated with glyphosate (360 g a.i. ha^{-1}); I, untreated; thickness of epidermis (TE), thickness of cortex (TC), diameter of stem (DS) diameter of central cylinder (pith) (DCC)

4. Conclusions

Glyphosate rate of 288 g a.i. ha^{-1} was able to recover alfalfa fresh fodder yield, as well as physiologic and anatomic parameters from detrimental effects of field dodder, demonstrating a photosynthetic success and growth of alfalfa plants. Also, the rate of 360 g a.i. ha^{-1} did not cause any adverse effects on alfalfa. Glyphosate is an herbicide that can help to solve field dodder problem in alfalfa crops. However, it should be kept in mind that treatments in this study were carried out at a very early stage of alfalfa growth. Later applications may not be as successful as earlier ones, which many other weed control studies have already indicated.

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Development of an Automatic System to Detect and Spray Herbicides in Corn Fields

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ABSTRACT
Weed control is vital in agricultural production. Chemical control methods are generally preferred in weed control as they (1) affect quickly and (2) reduce the labour requirement. However, in conventional applications chemicals are generally applied to whole field surface. Therefore, non-targeted areas are also sprayed. This increases 1) amount of herbicide used and (2) risk of off-target chemical movement. In this study, a patch spraying system was developed to automatically detect and spray herbicides on weeds in the corn field based on weed density. In order to determine the weed regions, a digital camera was fitted in front of the tractor. The images taken using the camera were then simultaneously processed using an algorithm written in Matlab^{TM} software. The results of the field study showed that at 4, 6 and 8 km h^{-1} forward speeds, application volumes decrease by 30.21%, 28.82% and 32.28%, respectively, when it is compared to the conventional application methods. It was also determined that the application accuracy rates were 80%, 81.66% and 75% respectively for 4, 6 and 8 km h^{-1} speeds.

Keywords: Patch spraying; Weed detection; Spraying application; Image processing

1. Introduction

Some weed species have gained resistance against regularly and intensively used chemicals. Therefore the amount of energy used for the weed control hence cost of weed control has increased. In corn production, product losses can be up to 20-30% in the first two months period from planting due to weeds. In addition, weeds in corn fields make harvesting difficult and cause work loss (Aydemir & Karaoğlu 2008). Weeds are not desired in the corn fields as they compete with corn for limited resources such as water, nutrients, light, and space. Weeds can also change the quality of light received by corn (Rajcan et al 2004).

Uncontrolled use of chemicals in weed control in agricultural production causes negative effects on the environment and human health. Therefore reducing the amount of chemicals used and increasing their effectiveness in agricultural production is vital to keep agricultural production sustainable. In order to overcome the negative economic and environmental risks of over application and reducing the amount of chemicals used, patch spraying has been suggested (Pajares 2015).

In order to apply patch spraying determination of the weeds in the field environment is essential. However, some weeds are randomly distributed on the field whereas some weeds might have patchy distributions. On the other hand crops are sown in rows with a constant spacing (about 70 cm for corn). On field images, these rows

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appear as parallel lines and crops are sown with a constant spacing between two plants in the same row (also called intra-row spacing) (Vioix et al 2006). The periodic distributions of these crops can provide significant advantages in terms of image processing applications as objects with specific shapes in an image can be identified easily.

In recent years, researchers have developed weed control systems based on various image processing techniques using different types of cameras and spectral sensors (Burgos-Artizzu et al 2011; Agrawal et al 2012; Hlaing & Khaing 2014). One of the methods used to determine the crop row is the Hough transform (HT) (Tang et al 2016). HT information proves to be a very good way to differentiate crop from weed pixels presenting similar spectral information (Ortiz et al 2015). On the other hand, the high computational time of HT's method is one of the disadvantages of this method for real-time applications. Sabzi & Gilandeh (2018) aimed to locate and identify potato plants and three common types of weeds using a hybrid classification approach, consisting of artificial neural networks (ANN) and particle swarm optimization algorithm (PSO). However, the speed of the developed method was too slow due to the excessive computation required to classify weeds. Gonzalez-de-Soto et al (2016) presented structure of a unmanned ground vehicle derived from the project RHEA (Robot fleet for highly effective agricultural and forestry management). For the control of the vehicle a hybrid architecture was implemented. The vehicle has a camera for real-time field vision, a GPS receiver to provide the position and orientation of the vehicle, a laser system placed in the front of the vehicle for obstacle detection and a smart spraying system for selective spraying application. Although the system can accurately detect and apply spraying, the system is quite costly and cannot be afforded by small scale farmers.

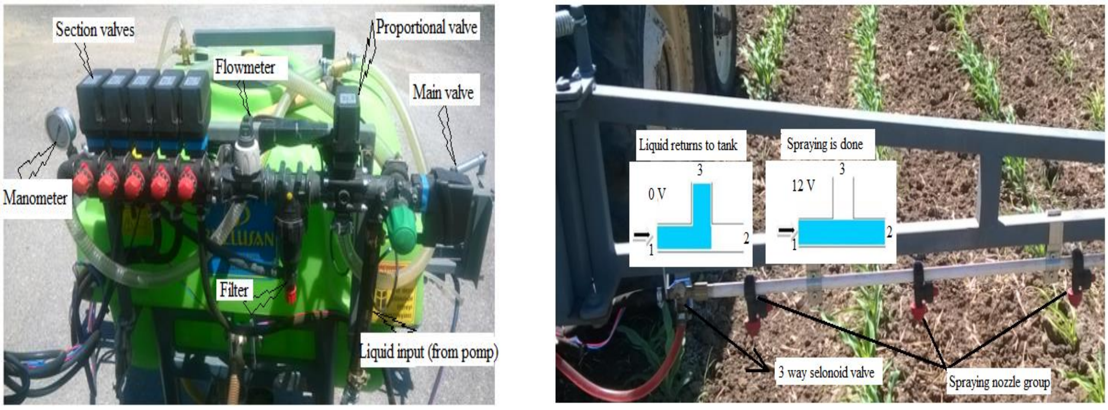
In this study, a small scale and cost effective patch spraying system was developed. Physical spraying applications were also performed using real time field images taken under uncontrolled outdoor lighting conditions. The aim of this study was to (1) determine real time crop-weed discrimination using morphological image processing techniques in corn field and (2) perform spraying application automatically to the desired area via a control system (if the weed density is greater than a determined critical level).

2. Material and Methods

2.1. Material

In this study, a 400 liter capacity three-point hitch type field sprayer was used. A camera and a speed sensor were mounted on an adjustable platform (Figure 1). The original sprayer regulator was removed from the system and replaced with a flow-based control unit (Figure 2a). This system was used only to prevent ripples in the pressure line because each nozzle group might be active at different times during the application. Section solenoid valves of the flow-based control system and three-way solenoid valves which activate the nozzle groups were connected to each other. The three-way solenoid valves were controlled by PLC (Programmable Logic Controller). When the sprayer was not used the liquid was sent back to the tank through a return line (Figure 2b). An IDS UI-1240ML-C-HQ camera; resolution of 1280 x 1024 pixels, 5.3 x 5.3 μm pixel-size and 6.784 mm x 5.427 mm optical size (IDS 2017) with an Azure model C-Mount, 1/2", 4 mm lens were used to acquire images. The camera was mounted on a custom-made height adjustable platform. To acquire 4.20 m. horizontal field of view (HFOV), different camera height (3-4 m) from the ground and different pitch angle (30°-50°) was used.

Figure 1- Sprayer system used in the study



(a) (b)

Figure 2- Flow-based control unit components (a) and spray nozzle group (b)

In order to measure the forward speed, a radar speed sensor (Dickey-John Radar III) was mounted in the front of the tractor. The supply voltage of the speed sensor was 12V DC whereas the output signal was a 12V square wave. The periodic signal of the 12V square wave coming from the radar speed sensor was increased to 24V amplitude by using a NPN type transistor so that this signal can be read by the PLC fast counter unit.

A Siemens brand PLC (model no: 1214C AC/DC/Rly) was used in the system. The PLC had 8 digital inputs, 6 digital outputs and 2 analogue inputs with 10 bit resolution. 6 digital inputs can be assigned as a fast counter (HSC) input to read signals up to 100 kHz.

2.2. Methods

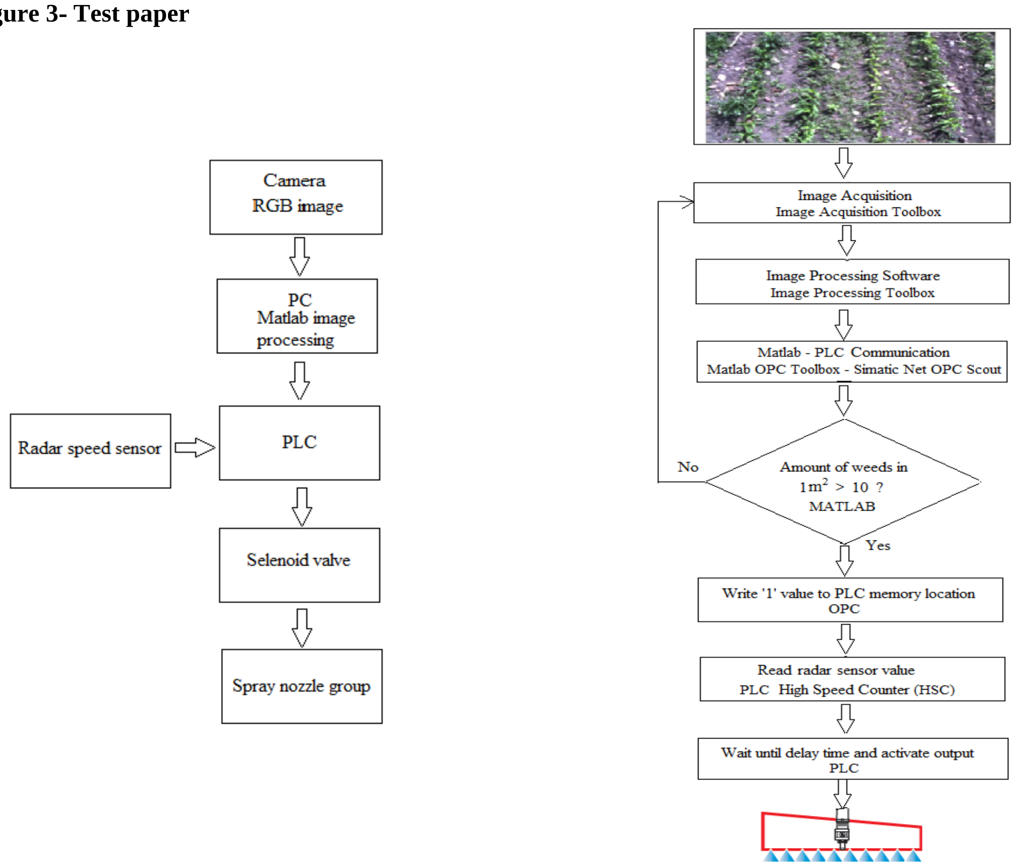
This study was carried out in four stages namely; (1) transferring field images to Matlab^{TM} and processing them to classify corn plants and weeds, (2) transferring data from Matlab^{TM} to PLC via OPC (Ole for Process Control) server, (3) calculation of the spraying delay times using the PLC program developed at 'Tia Portal' (Totally Integrated Automation Portal) in accordance with the information from the radar speed sensor, and (4) application of spraying to the required areas.

In order to check if the system is working, firstly, some preliminary tests were performed. After the preliminary tests, the system was then tested under the field conditions. To determine the accuracy of the field test applications, 20 times 0.05 x 0.06 m white papers were placed at different points on the land. To identify the droplets sprayed on to the papers some red food colouring was also added to the sprayer tank (Figure 3). These reference papers were placed in high and low dense weed areas and the accuracy of spraying application was analysed in terms of droplets presence (only by visually observing the presence of droplets) on the papers. For example, if the patch spraying was successfully applied 16 of the 20 papers, the accuracy rate was accepted as 80%. Field tests were conducted to examine two different situations (which have been explained below);

a. Conventional spraying application: In order to determine the application norms for traditional application at different tractor speed (Camera data was not used in this application).

b. Patch spraying application (Depending on the camera data): Unlike the conventional application, the patch spraying application was performed using camera data. After the image was taken, firstly, a region of interest (ROI, 1.5 x 4.20) which is equal to working width of the sprayer boom was determined. After that the ROI was divided into sub regions automatically. Subsequently, the weed regions in the sub-sections were compared to a pre-determined weed threshold value. If the sub-sections had more weed than the threshold value, spraying was applied. In the sub-regions where the weed amount is less than the specified threshold value, the spraying process was interrupted and the liquid was returned to the tank through solenoid valve. The block diagram of the automatic controlled field sprayer system and the general algorithm of the system were given in Figure 4a and 4b, respectively. Algorithm steps include processes from image acquisition to spraying application.

Figure 3- Test paper



(a) (b)

Figure 4- a, Block diagram of control system; b, algorithm used in the system

Images with a resolution of 752 x 480 pixels obtained by the camera were transferred to Matlab^{TM} environment via Image Acquisition Toolbox™. Image Acquisition Toolbox™ provides functions and blocks that enable to connect industrial and scientific cameras to Matlab^{TM}. It includes Matlab^{TM} applications that interactively detect and configure hardware properties (Matlab 2017). After transferring the images to Matlab^{TM} environment, the RGB (Red, Green, and Blue) images were, firstly, converted into the grayscale images. In a colour image, a pixel value consists of different combinations of Red (G), Green (G), Blue (B) values. Converting of the colour image into grayscale was to make the brightness value of the green objects (crops and weeds) greater than the other objects in the image (soil, stone, etc.), which will increase the accuracy in the binary level conversion phase. An example of how an RGB image taken from the field was converted into a grayscale image was given in Equations 1, 2, 3 and 4. Firstly, each field image was converted to normalized red (R), green (G), and blue (B) channel images. Then the normalized RGB channels were converted to the normalized excessive green (NEG) images to emphasizing green channel (Jeon et al 2011). This transformation was firstly employed by Woebbecke et al (1995). Then similar equations were used in several studies (Sabancı 2013; Hlaing & Khaing 2014; Liu et al 2014).

$$NEG = 2*G-R-B \tag{1}$$

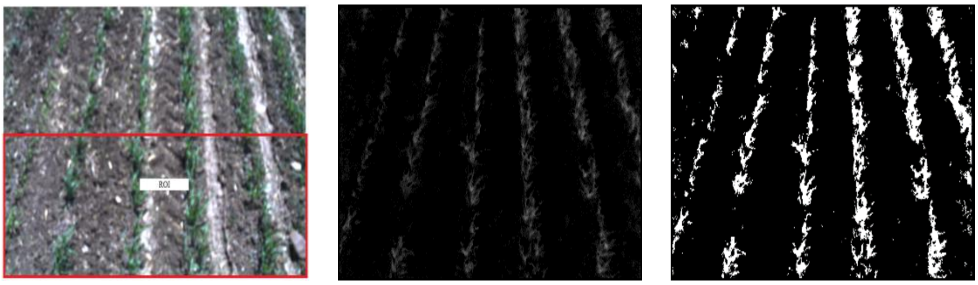
$$R = (r)/(r+g+b) \tag{2}$$

$$G= (g)/(r+b+g) \tag{3}$$

$$B= (b)/(r+b+g) \tag{4}$$

Where; r, g, and b are a pixel value of red, green and blue channel of RGB image.

In order to convert *NEG* images into binary images thresholding method was used. Although there are different thresholding method available in the literature, such as histogram-shaped-based, clustering-based, entropy-based, attribute similarity methods, object attribute-based, spatial approaches and local methods (Sezgin & Sankur 2004). In this study "Otsu automatic threshold method", which chooses the threshold to minimize the intraclass variance of the black and white pixels, was used due to its simplicity (Otsu 1979). The pixels below the threshold value were considered as black (soil, stone and other materials) while pixels above the threshold value was considered as white (corn and weed). An example of converting of an RGB image to binary image was given in Figure 5.



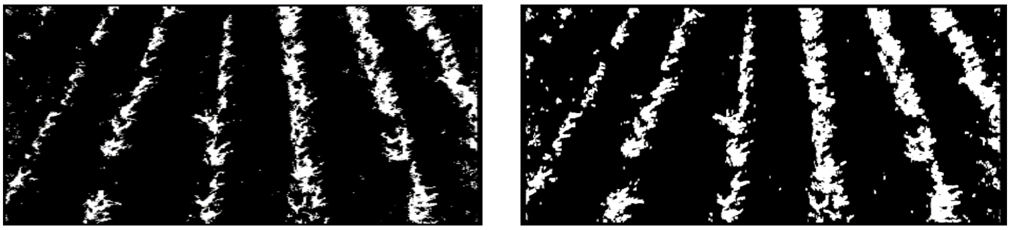
a b **c**

Figure 5- a, Original image; b, gray level image; c, binary image

In order to establish the communication between Matlab™ and PLC, OPC Toolbox functions (available in Matlab™) were used. To do so, firstly, the OPC connection settings between the PLC and the PC were made using the OPC.Simatic.Net software. After that Matlab™ OPC Toolbox has been assigned as a client, and thus Matlab™ had information about the server's name and each OPC item stored in the server (Tekinalp et al 2013). Then OPC group object was created and added into OPC items which represent the PLC memory.

PLCs require special equipment to detect signals faster than its cycle time. Therefore one of the PLC inputs was assigned as a fast counter to read the information sent by radar speed sensor. The S7-1200 PLC used in this study had six high-speed counters and these channels can be used in the 'Tia Portal' software to read signals up to 100 kHz. The radar speed sensor was connected to the I0.0 input of the PLC and the speed information was transferred to the 'HSC_1' channel. Spraying application delay time was calculated using the tractor speed information and the constant distance between the image frame and the spray nozzles (4.60 m). When the boom came to the weed area, which was sensed by the camera, spraying application started and continued along 1.5 m.

While corn rows showed a regular arrangement in the vertical direction, there was no regular arrangement for weeds. In order to process the images, firstly, the noise pixels (smaller than 5 pixels) on the image were cleaned. Then, in order to merge the pixels of the crop rows, extending vertically in the binary image, each image was dilated using a 1 pixel wide and 9 pixels long structuring element. The reason of using only one pixel wide structuring element was to prevent the incorporation of crop rows with weeds. As corn crops were arranged in equal intervals in the vertical direction, the possibility of structuring element to merge the crop rows was greater than that of the weeds. However, it should be noted that the irregularities in corn plant rows (deviations from sowing errors) were neglected. The binary image and its dilated status by structuring element was shown in Figures 6a and 6b.



(a) (b)

Figure 6- a, Original binary image; b, dilation of the binary image with a 9x1 structuring element

After the dilation process, there was a clear difference between the merged crop rows and the weeds areas. In order to eliminate crop rows from the image a threshold value, calculated using Equations 5, was applied.

$$Object = \begin{cases} Weed & area < 1200\, pixels \\ Crop & area \geq 1200\, pixels \end{cases} \quad (5)$$

Figure 7 shows the location of region of interest (ROI) from the processed image where crop rows and weed pixel groups displayed in different colours. In the field tests, dimension of the ROI was determined as 4.2 m wide and 1.5 m vertical long. The width of the ROI area was determined considering the coverage of the nozzles. After generating the binary image (which contains only classified objects (crops and weeds)), the objects representing crop rows were removed from the binary image and the image containing only weeds was divided into three sub-sections (the same size as each nozzle group) vertically (Figure 8). The spray nozzles were also divided into three sections, using the same measurements, as right (section 1 with 2 nozzles), middle (section 2 with 3 nozzle) and left (section 3 with 2 nozzle) and each section was independently controlled through three-way solenoid valves. Then spraying was applied to each region in terms of the amount of weed per m^2.

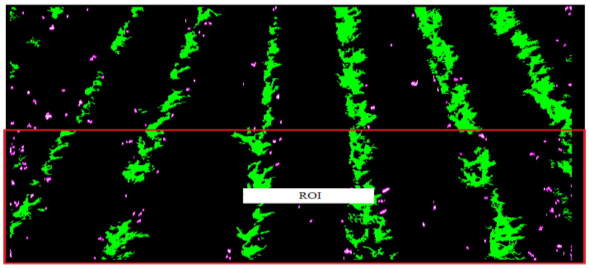


Figure 7- Location of region of interest (ROI) with corns and weeds (in colour)

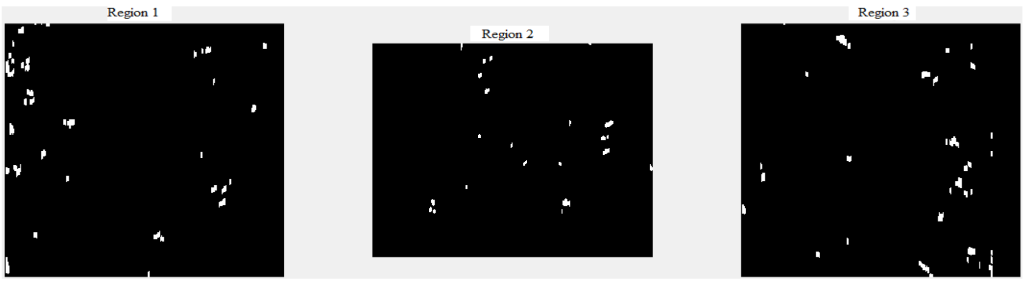


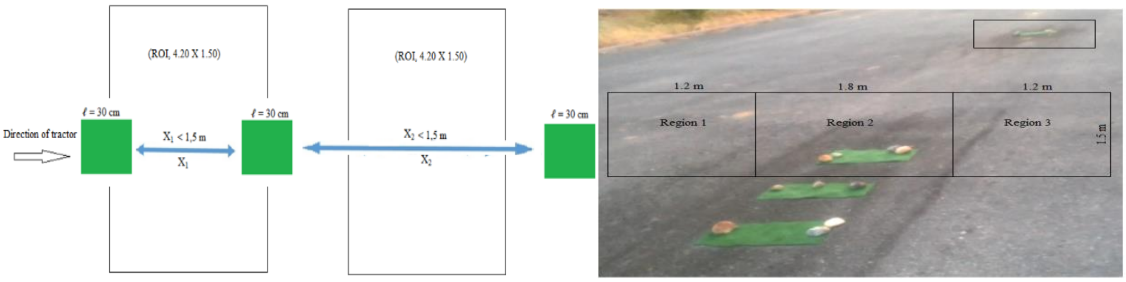
Figure 8- Dividing the ROI into three sub-sections using binary image that only contains

According to TAGEM (2017), for species with known damage threshold, the weed density must be less than the least damage threshold, whereas in the case of species whose damage threshold is unknown, the damage threshold was determined as 10 pieces in per m² or 10% of the area. Üstüner & Güncan (2002) classified the weed densities as; A) very dense (average> 10 m^{-2}), B) dense (average= 1-10 m^{-2}), C) medium dense (average= 0.1-1 m^{-2}). In this study, spraying was applied, if the amount of weed in m^2 in each image was equal to or greater than 10 (very dense).

3. Results and Discussion

3.1. Preliminary tests

In the first stage, preliminary tests were performed by placing green objects at different spacing on a flat concrete surface to investigate whether or not the spraying application was performed at the right time and to the right regions. Since the ROI has a width of 1.5 m in the direction of the tractor travel, if the distance between the objects is less than 1.5 m (X_1), the spraying process continues without interrupting between the two objects. If the distance between objects is more than 1.5 m (X_2), spraying is done only the areas where the objects are located (Figure 9a and 9b).



(a) (b)

Figure 9- Conditions for spray application for preliminary test, (a) a preliminary test (b)

During the tests, OPC item values were read at intervals of 0.2 s by changing the tractor speed and the distance between the green objects. For example, when the green object in the section 2 of the image area is detected during the application, the reading process of the PLC memory area is shown below.

```
valve2 =
        ItemID: 'S7:[S7_connection_1]M1.3'
        Value: 1
        Quality: 'Good: Non-specific'
        TimeStamp: [2017 7 7 19 27 54.6280]
        Error: '
```

Time difference of spraying between green objects are given in Table 1 for 4 km h^{-1} tractor speed and 2 m distance between the objects. As seen in Table 1, spraying application was performed at time intervals of about 1.5 s to the areas where the green objects were located in the display area. In other words, the areas between the objects were not sprayed for approximately 1.5 s.

Table 1- Moments of spraying onto objects during a preliminary test

Tractor speed and distance between objects	*Object no*	*Item ID*	*Application start times for each object*	*Time difference (ms)*
4 km h^{-1}, 2 m	1	'S7:[S7_connection_1]M1.3'	[2017 7 12 20 5 40.3350]	0
	2	'S7:[S7_connection_1]M1.1'	[2017 7 12 20 5 41.9130]	1578
	3	'S7:[S7_connection_1]M1.3'	[2017 7 12 20 5 41.9240]	11
	4	'S7:[S7_connection_1]M1.5'	[2017 7 12 20 5 41.9410]	17
	5	'S7:[S7_connection_1]M1.1'	[2017 7 12 20 5 43.5530]	1612
	6	'S7:[S7_connection_1]M1.5'	[2017 7 12 20 5 43.5770]	24

3.2. Field tests

Field tests were performed within the critical period for weed control (for corn), which was between the 20th and 55th day after planting (Tursun et al 2015). Spray applications were performed using water at 4, 6 and 8 km h^{-1} forward speeds in a 250 m long and 20 m wide area (Figure 10).

Figure 10- Field tests

Firstly, the accuracy of the field tests were determined using patch spraying application method depending on the camera data at 4, 6 and 8 km h^{-1} speeds. Accuracy test results obtained by looking at the presence of droplets on test papers placed on the application land. Results are given in Table 2. It was found from the results that the accuracy of the tests performed at 8 km h^{-1} was lower than those of the 4 and 6 km h^{-1} ones. This can be attributed to the vibration (which increased with the increase of the speed) which reduced the quality of the images hence the performance of the sprayer.

Table 2- Accuracy rates of applications with test papers

Speed (km h^{-1})	*Repeat*	*Correct applications*	*Incorrect applications*	*Average accuracy rate %*
4	1.	16	4	80.00
	2.	15	5	
	3.	17	3	
6	1.	17	3	81.66
	2.	16	4	
	3.	16	4	
8	1.	14	6	75.00
	2.	15	5	
	3.	16	4	

In the second step, the water volumes applied in conventional spraying application and patch spraying application method based on camera data were examined at 4, 6 and 8 km h^{-1} forward speeds. Comparison of the application volumes for both methods were given in Table 3. Results showed that when it is compared to conventional method 30.21%, 28.82% and 32.28% less water was used in the patch spraying application method based on camera data at 4, 6 and 8 km h^{-1} operating speeds, respectively. Data given in Tables 2 and 3 showed that a more effective spraying can be applied using patch spraying application method based on camera data at low operating speeds (4 and 6 km h^{-1}).

Table 3- Comparison of application volumes of classical and camera-based patch spraying methods

Speed (km h^{-1})	*Repeat*	*Convensional application method*		*Patch spraying method depending on camera data*		*Difference (%)*
		Applied volums (L)	*Avarage (L)*	*Applied volums (L)*	*Avarage (L)*	
4	1.	37.34	37.41	24.84	26.11	-30.21
	2.	37.50		26.20		
	3.	36.40		27.30		
6	1.	25.20	25.33	17.00	18.03	-28.82
	2.	26.00		18.70		
	3.	24.80		18.40		
8	1.	19.20	18.46	12.70	12.50	-32.28
	2.	18.50		11.60		
	3.	17.70		13.20		

In recent years, there has been a notable increase in studies regarding to the control of weeds using different digital image processing software (i.e., Matlab, Open CV, C++) (Burgos-Artizzu et al 2011; Vikhram et al 2018). Some factors that negatively affect the performance of these systems and the current system were that (1) vibration (which distorts the image quality), (2) sun rays (particularly in sunny days, infrared radiation enters the sensor impacting the different spectral channels coming from different angles (Romeo at al 2013) and (3) radar speed sensor output frequency sensitivity (due to the dense vegetation). The efficiency of the systems can be increased by analysing smaller areas using two or more cameras at a lower distance (to reduce camera vibration in field conditions). It is thought that the efficiency of the system can be improved by using more comprehensive image processing algorithms and enhanced computational power.

4. Conclusions

In this study, a system was developed to automatically determine weeds in a corn field and perform spray application (if the weed density is greater than a critical level). Field tests were performed to evaluate the efficiency of the system and it was found that the accuracy of patch spraying application method using camera was at 80%, 81.33% and 75% for 4, 6 and 8 km h^{-1} operation speed, respectively. In order to improve the success of the system infrared-cut filters, which help to reduce the sun light reflected by the corn leaves, can be used (Romeo at al 2013). It is also thought that by using the proposed system (1) negative effects of the chemicals used in agriculture on environment and human health and (2) the production costs can be reduced. Future work will focus on improving (1) the algorithm to increase accuracy of the image analysis and (2) the system to improve its effectiveness.

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Use of Super Absorbent Polymers with *Euonymus* Plants (*Euonymus japonicus 'Aureomarginatus'*) in Ornamental Plant Cultivation

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ABSTRACT
In this study, the usability of super absorbent polymers (SAP) and its effects on water consumption, irrigation and labor costs and plant growth in ornamental plant cultivation were investigated by using *Euonymus japonicus 'Aureomarginatus,'* peat, river sand, and Wesoorb branded SAP. For a period of 152 days, the growth of the control groups without SAP and experimental groups with SAP was monitored, and the obtained results were compared. It was determined that SAP balanced the moisture of the medium with a controlled release and decreased the water stress in the plant and differentiated the root structure. The use of SAP in *Euonymus japonicus 'Aureomarginatus'* cultivation reduced water use by 45% on average and labor costs by 48% on average. It was observed that SAP can be used in ornamental plant cultivation and will decrease production costs.

Keywords: Ornamental; Hydrogel; Sustainable; Water; Growth; *Euonymus*

1. Introduction

First synthesized in the United States towards the late 1950s, super absorbent polymers (SAPs) entered into industrial use in Europe and Japan in the 1970s (Trijasson et al 1990). Owing to their crosslinks, SAPs are insoluble in water, and can absorb 10 to 1.000 times their weight in water, saline water or physiological liquids (Pó 1994; Bhagat et al 2016). Their areas of use include diapers and disposable underpads (Trijasson et al 1990), wastewater treatment (Dhodapkar et al 2007; Bhagat et al 2016), hygienic products, sensors (Chen et al 2004), the pharmaceutical and drug sector (Mo et al 2006), the food sector (Casquilho et al 2013; Bhagat et al 2016), bioengineering (Bai et al 2013), biomedical technologies (del Valle et al 2017; Chatterjee et al 2018), and agricultural technologies (Mo et al 2006; Moslemi et al 2011; Bai et al 2013).

Potassium-containing polyacrylic- and polyacrylamide-based SAPs are widely used in agricultural applications (Ekebafe et al 2011; Moslemi et al 2011). Studies into SAP have investigated its ecotoxicological effects, revealing no toxic effect on water and soil (Madakbaş et al 2014; Bhagat et al 2016). In contrast, it has been reported that a controlled application could improve or control microbial activity (Li et al 2014). Previous studies have suggested that SAP can accelerate plant growth by promoting fertilizer retention (İsmail & Kuyulu 2003), and that a plant's level of interaction with its environment varies according to the quantity of administered SAP, which in turn increases product yield by reducing water-based stress (Sayyari & Ghanbari 2012). It has been also reported that

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SAP increases plant diameter, size, leaf number and leaf width; boosts shoot formation and root growth; and extends plant lifespan (Ruqin et al 2015; Souza et al 2016). Its use on seeds normally involves covering seeds with SAP or germinating them within SAP, while use on plant roots involves application of SAP in gel form (Madakbaş et al 2014). In the literature reviews, it was determined that the studies were generally carried out on fruit and vegetable cultivation (Madakbaş et al 2014) and increase of product yield (Sayyari & Ghanbari 2012), similar studies have been conducted on ornamental plants such as tropical ornamental plants (Wang 1989), coarse-structured garden plants (Fonteno & Bilderback 1993), *Cupressus arizonica* (Koupai & Asadkazemi 2006), tree seedlings (Orikiriza et al 2013), ornamental *Salvia* species (Ljubojević et al 2017), but there is no study conducted on *Euonymus japonicus* which is frequently used in plant design applications (Gül et al 2006; Eren & Var 2016) and has high water needs (Gül et al 2006).

In this study, it was aimed to investigate the effects of SAP usage on water consumption, irrigation and labor costs, plant shoot and root growth in *Euonymus japonicus ‘Aureomarginatus’* cultivation environment. For a period of 152 days, the growth of the control groups without SAP and experimental groups with SAP was monitored, and the results were compared to determine the effects of SAP on water usage, costs, and plant growth.

2. Material and Methods

2.1. Preparation of materials and samples

The peat and *Euonymus japonicus ‘Aureomarginatus’* used in the study trials were commercially supplied by Göker Agriculture (Yalova, Turkey), while the Wesoorb-branded SAP was supplied by Ecotech Co. Ltd. (Izmir, Turkey). For each pot, Wesoorb-branded SAP in powder form was manually added to peat or river sand in the quantities indicated in Table 1 and mixed thoroughly to ensure equal distribution. *Euonymus japonicus ‘Aureomarginatus’* seedlings were planted in 1.5 L flowerpots taking into account the maximum water holding capacity of SAP and leaving a 20% irrigation gap from the top surface, and they were labeled and taken into the greenhouse environment. HUBO-branded UX100-003 temperature and humidity dataloggers (temp/RH loggers) placed at different points provided continuous monitoring of the temperature and humidity in the greenhouse.

Table 1- Parameters used in the study

Code	*Peat (g)*	*River sand (g)*	*SAP (g)*	*Number of plants*
R-1	1.200	-	-	45
R-2	-	1.200	-	45
TS-1	1.200	-	0.8	45
TS-2	1.200	-	1.0	45
TS-3	1.200	-	1.2	45
KS-1	-	1.200	0.8	45
KS-2	-	1.200	1.0	45
KS-3	-	1.200	1.2	45

2.2. Characterization

The IR spectrum of the SAP additive in pellet form was taken at room temperature using a Perkin Elmer Spectrum 100 FT-IR device, with the aim of determining the groups attached to the monomer units. An Olympus BX51M optical microscope was used to measure the particle size of the SAP additive so as to determine average particle size and surface morphology. The determination of the water retention capacity (Q) of SAP, as well as the absorption test (Bakass et al 2002; İsmail & Kuyulu 2003; Bai et al 2013) and the desorption tests (İsmail & Kuyulu 2003; Mo et al 2006; Bai et al 2013) of peat and sand were all carried out as described in literature.

The level of moisture of the cultivation medium was measured daily with a Tartes Decagon-01-branded digital soil moisture meter. After planting, the pots were weighed, and their initial conditions were recorded. At first, irrigation was performed from the top in a way to wet the entire surface of the soil in order to determine the water holding capacity of the medium. At the point when the water started to flow through the drainage holes, irrigation was stopped, and the pots were weighed again. By using similar studies in the literature (İsmail & Kuyulu 2003), the maximum water capacity that the soil can hold without drainage was determined by comparing the measured weight with the initial weight. Similarly, to the study carried out by Gao et al (2013), different ratios of water between 20% and 25% of the volume of the root medium, 500 mL for each pot, was given for the formation of adequate drainage and for determining the amount of irrigation (Connellan 2002; Gao et al 2013; Varış 2017). An attempt to determine the optimum amount of irrigation was made by performing drainage measurements and weighing the pots.

The seedling size and dry root weight measurements performed before planting were repeated at one-month intervals, to monitor plant growth on a monthly basis. The plants were pruned at the end of the second and fourth months, and the pruned plant parts were weighed on a precision scale to determine the effect of SAP on shoot formation.

2.3. Calculation of costs

Formula number 1 was used in the calculation of the total amount of water used (*TW*) depending on the amount of water used in irrigation (*W*) and the number of irrigation repetitions (*Q*) by determining from drainage measurements. While the cost of water used (*WP*) was calculated from formula number two, irrigation labor costs (*LC*) and total cost (*TC*) were calculated from formulae number three and four, respectively. The unit water prices for public institutions of Yalova Municipality (*UP*) were used as a basis in the calculation of *WP*. The seasonal worker wage (*dLC*) depending on the number of days worked was used in the calculation of *LC*. The studies of Ahmed et al (2013), Kim et al (2015), Kumar (2015) and Landscheidt & Kans (2016) were used for the formulae used in cost calculations.

$$TW\ (L) = W\ (L) \times Q \quad (1)$$

$$WP\ (₺) = TW\ (L) \times UP\ (₺/L) \quad (2)$$

$$LC\ (₺) = Q \times dLC\ (₺) \quad (3)$$

$$TC\ (₺) = WP\ (₺) + LC\ (₺) \quad (4)$$

The amounts of savings in labor costs and water usage were determined by proportioning the data obtained from the control group to the data obtained from the experimental group. The results obtained from cost calculations were compared with the results of similar studies in the literature (Nnadi 2012; Madakbaş et al 2014; Bhagat et al 2016).

3. Results and Discussion

3.1. FT-IR spectrum

Figure 1 shows the IR spectra of the acrylamide and potassium acrylate copolymers. The peak observed at the 1691 cm^{-1} wavelength corresponds to the hydrogen bond made by an NH_2 group in the acrylamide structure (Chen et al 2004). The peaks between 1400 cm^{-1} and 1600 cm^{-1} are possibly stretching vibrations associated with CH_2. The peaks near the 1100 cm^{-1} wavelength correspond to C=O bond stretching in the carboxyl acid structure. The aliphatic C-H stretching vibrations within the structure are observed between the 2700-2929 cm^{-1} wavelengths (Durukan 2007). The IR data suggests that the monomeric units are bonded in a graft copolymer arrangement (Chen et al 2004).

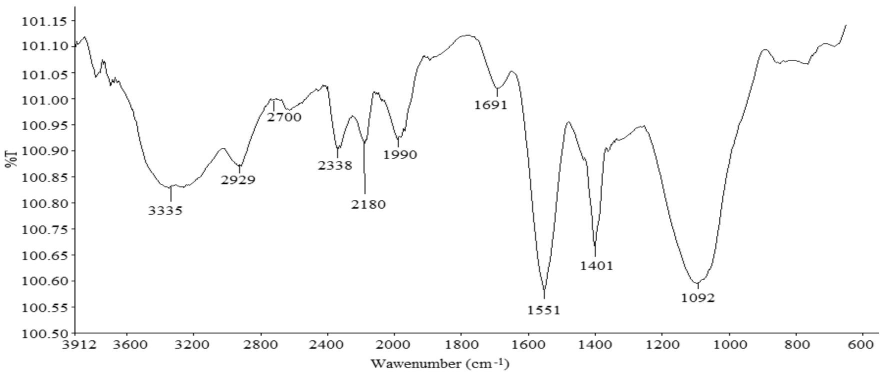

Figure 1- IR spectra of acrylamide and potassium acrylate copolymer

3.2. Optical microscope examination

The average particle size of SAP, whose optical microscope image is shown in Figure 2, was calculated as 150 µ. The particle sizes varied between 80 and 250 µ, and this variation is assumed to have an effect on water retention capacity. Trijasson et al (1990) described similar results in their study.

Figure 2- Stereomicroscopy image of particles

3.3. Characterization of the SAP additive

The water retention capacity of SAP when dry was found to be 250 times its weight, while its water absorption speed was 8-15 minutes. Absorption occurred rapidly during the first eight minutes, reaching a steady pace in approximately 40 minutes. The SAP absorption graph and desorption graph are presented in Figures 3.a and 3.b, respectively. The soil contraction that formed depending on the SAP ratio and water loss resulted in a pressure on SAP that accelerated the rate of desorption. İsmail & Kuyulu (2003), Mo et al (2006) and Bai et al (2013) described similar results in their study.

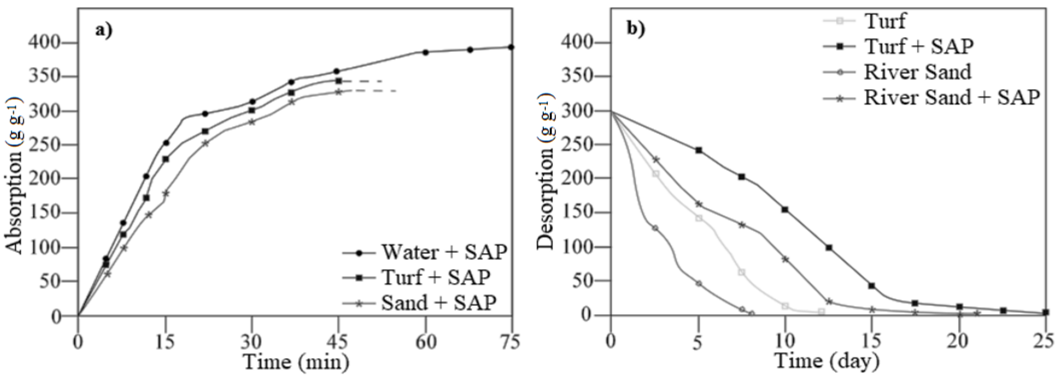

Figure 3- a) SAPs Absorption and b) SAPs Desorption graph

The medium designated as TS-3 had the longest intervals between watering, with 22 days between each watering, while the medium designated as R-2 had the shortest interval, with six days between each watering. The SAP ratios, the watering intervals according to the cultivation medium, and the amounts of water used over the 152 days are shown in Table 2.

Table 2- The amount of water used in the study

Code	*Interval between each watering (d)*	*Number of watering*	*Amount of water used (mL)*
R-1	10	15	5.000
TS-1	17	9	3.200
TS-2	20	7	2.600
TS-3	22	6	2.300
R-2	6	25	8.000
KS-1	9	16	5.300
KS-2	11	13	4.400
KS-3	14	11	3.800

3.4. Determination of water holding capacity and the amount of irrigation water

Water holding capacity without drainage was calculated to be 240 mL for turf and 108 mL for sand. It was observed that similar results were obtained with the calculations made by Connellan (2002). It was determined that the amount of 300 mL irrigation water, which is 25% of the total root media, provided adequate drainage in the pot and was the ideal amount of water to be used in irrigation periods. The results obtained from drainage measurements were observed to be consistent with the studies aimed at determining the adequate amount of drainage in pot plants (Varış 2017).

3.5. Humidity measurement results

Relative humidity inside the greenhouse was 55-60 percent in the morning and 90-95 percent at night, while the average temperature values were 25 °C in the morning and 17 °C at night.

The daily soil moisture measurements showed that the water absorbed by SAP - depending on the time, ambient temperature, evaporation and soil moisture loss - is later released, ensuring that the soil remains moist (İsmail & Kuyulu 2003; Casquilho et al 2013). In a peat-based medium, SAP formed tiny islets that provided humidity by releasing previously absorbed water at a balanced rate. The structure of peat ensured that the medium stayed humid for longer periods. In the sandy media, the sand particles carried the water absorbed by SAP into the medium. It is thought that the speed at which water is carried in a sandy medium affects its ambient humidity, and that this leads to greater humidity loss when compared to the fully peat medium (Chen et al 2004). The soil humidity measurement results, which were taken daily and determined using weighted averages, are given in Table 3. The results indicate that humidity, which has an important effect on the environment/medium, can be controlled by using SAP; that SAP will prevent the unnecessary use of water to ensure the effective use of irrigation systems; and that the environment/medium has a significant effect on humidity. Chen et al (2004) and Souza et al (2016) reported similar results in their study.

The increased addition of SAP to a peat-based medium decreased the level of humidity within the medium, while adding more SAP to a sand river-based medium increased the level of humidity within the medium. The decrease in humidity in the peat medium was related to the water retention capacity of peat, with the water held inside the peat being absorbed by the SAP. On the other hand, adding SAP to a sandy medium resulted in the medium remaining more humid, depending on the amount of SAP used (Chen et al 2004). Measurements performed after irrigation indicated that the SAP allowed both types of medium to remain humid for longer. The peat medium remained humid for longer, despite watering at greater time intervals.

Table 3- Moisture content of soil

	Moisture content of soil (%)							
Day (d)	*R-1**	*TS-1**	*TS-2**	*TS-3**	*R-2**	*KS-1**	*KS-2**	*KS-3**
1	37.4^{a}	38.1^{b}	37.8^{a}	37.5^{a}	22.0^{a}	22.5^{b}	23.0^{c}	24.4^{a}
2	34.8^{a}	36.8^{a}	36.7^{c}	36.6^{c}	18.4^{e}	20.5^{b}	21.2^{a}	23.1^{b}
3	32.6^{c}	35.4^{b}	35.6^{a}	35.7^{c}	14.7^{f}	17.4^{a}	19.5^{b}	21.7^{a}
4	29.8^{d}	33.9^{b}	34.6^{b}	34.7^{a}	11.1^{a}	16.6^{c}	17.6^{c}	20.1^{b}
5	27.4^{b}	32.5^{b}	33.5^{a}	33.8^{d}	7.1^{b}	14.7^{d}	15.8^{a}	18.9^{c}
6	24.7^{b}	31.1^{c}	32.5^{b}	32.9^{f}	3.8^{a}	12.6^{d}	13.9^{a}	17.5^{d}
7	21.9^{c}	29.8^{e}	31.4^{d}	31.9^{b}	-	10.7^{b}	12.1^{d}	16.0^{a}
8	19.6^{e}	28.4^{d}	30.3^{c}	31.1^{d}	-	8.8^{e}	10.4^{a}	14.7^{c}
9	16.7^{f}	26.9^{c}	29.4^{b}	30.2^{a}	-	4.7^{c}	8.6^{e}	13.3^{c}
10	14.4^{a}	25.5^{c}	28.3^{a}	29.2^{a}	-	-	6.8^{a}	11.9^{b}
11	-	24.1^{c}	27.1^{d}	28.3^{c}	-	-	5.2^{d}	10.5^{e}
12	-	22.7^{b}	26.2^{b}	27.4^{c}	-	-	-	9.1^{a}
13	-	21.3^{c}	25.0^{b}	26.5^{e}	-	-	-	7.7^{e}
14	-	19.9^{e}	23.9^{c}	25.5^{b}	-	-	-	6.3^{d}
15	-	18.5^{f}	22.9^{d}	24.6^{b}	-	-	-	-
16	-	17.0^{b}	21.7^{a}	26.7^{a}	-	-	-	-
17	-	15.5^{a}	20.5^{b}	22.7^{d}	-	-	-	-
18	-	-	19.6^{b}	21.8^{b}	-	-	-	-
19	-	-	18.4^{c}	20.9^{c}	-	-	-	-
20	-	-	17.6^{b}	19.9^{d}	-	-	-	-
21	-	-	-	19.0^{c}	-	-	-	-
22	-	-	-	18.2^{e}	-	-	-	-

*, P<0.05; numbers in the same column with the same letter are not significantly different

3.6. Effects on plant shoot development

During planting, the average plant size was measured as 11 cm. A graph indicating plant size growth, based on measurements performed in succeeding months, is shown in Figure 4. The shoot size measurements made in the first month in both the control and trial groups gave higher values when compared to the following months. This is believed to have stemmed from the plant’s transition from the seedling medium to the richer and larger cultivation medium, and also from the fact that this period coincided with the start of the vegetation period. A comparison of the control and trial groups revealed that plants in the SAP-added media exhibited greater total size growth than those in the control groups. This observation can be explained by the balanced humidity that SAP ensured in the medium until the next irrigation time, which reduced plant water stress and promoted plant growth (Chen et al 2004; Casquilho et al 2013).

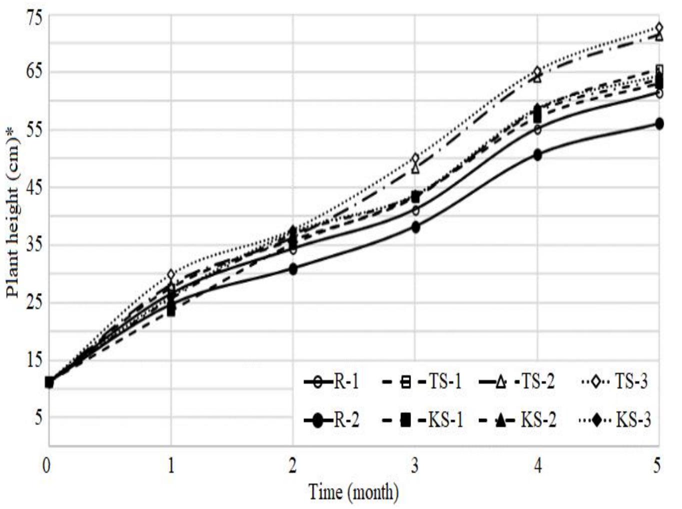

Figure 4- Plant height development chart (*P≤0.05)

In both growth media, an increasing quantity of SAP resulted in increased plant size. The best size growth was obtained in the peat media with SAP added. On the other hand, the level of plant growth in river sand media with SAP was similar to the one observed in the peat media without SAP. SAP added to river sand promoted plant growth by maintaining the absorbed water within the medium and preventing rapid evaporation (Moslemi et al 2011).

An effect similar to the one on shoot size was also observed on shoot growth density, which is measured based on shoot size, number of shoots and stem thickness. The size, biomass weight and growth density of the pruned plant parts are presented as a graph in Figure 5. The best growth density was seen in the trial group designated TS-3, and plant growth in the peat+SAP cultivation medium was generally measured as being better when compared to the river sand+SAP medium. Growth density in the trial groups was higher than in the control groups. In both types of cultivation media, the higher the addition of SAP, the higher the plant growth density was (Li et al 2014). Compared to the control samples, SAP addition accelerated plant growth without altering the plant growth balance. The fact that the plant growth density remained the same despite the increase in plant size, leaf and shoot number, and in the biomass of pruned plant parts suggested that the plants' growth remained controlled and balanced. This is further confirmed by the fact that in both the control and trial groups, the growth density at the end of the second and fourth months were at the same levels as in the preceding months. The humidity balance also affected plant growth, with higher levels of humidity that lasted longer resulting in higher plant growth density (Casquilho et al 2013).

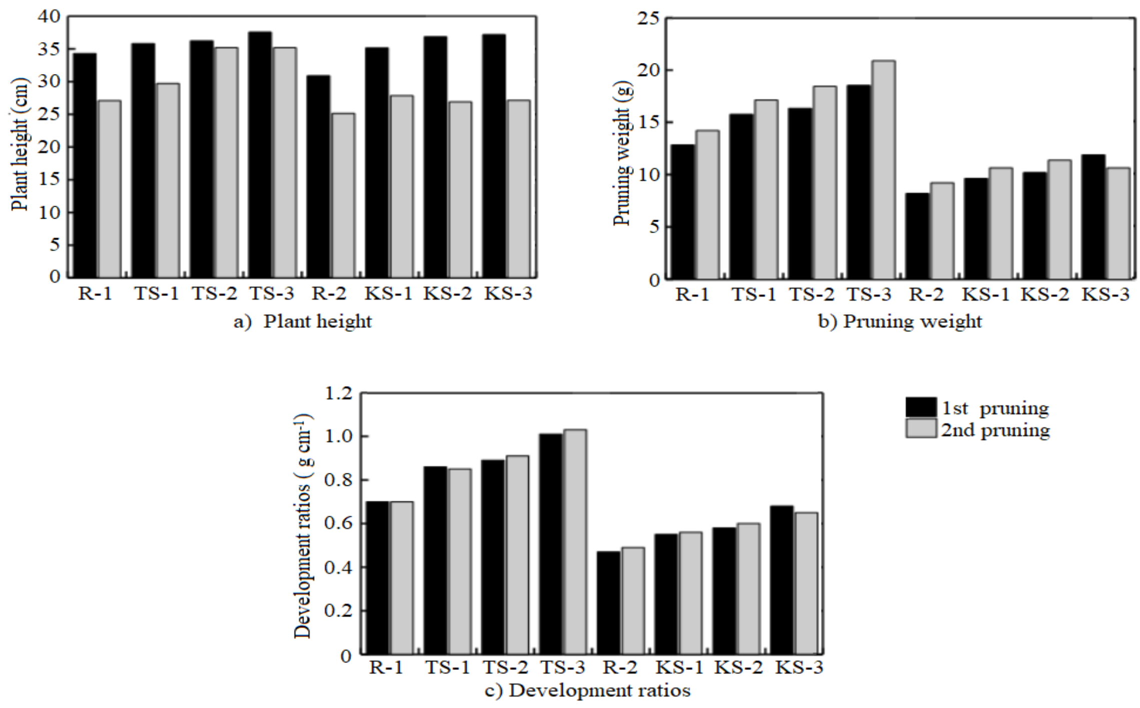

Figure 5- Height, weight and development ratios after pruning

3.7. Effects on plant root development

The average pre-planting root weights of the seedlings was 8.5 g. Figure 6 shows the five-month development of root weight. The monthly measurements revealed that the intervention group had a higher dry root weight than the control group. *Euonymus japonicus* plant roots in a peat medium were less numerous yet thicker when compared to the sand medium, while roots in the sand medium were more numerous yet thinner compared to the peat medium. This suggested that the sandy medium caused the plant to form more roots in different directions in order to reach water (Figure 7). The data presented in Figure 6 and Figure 7 show that while SAP had an effect on rooting, actual root formation varied according to the type of medium and the amount of SAP (Sayyari & Ghanbari 2012).

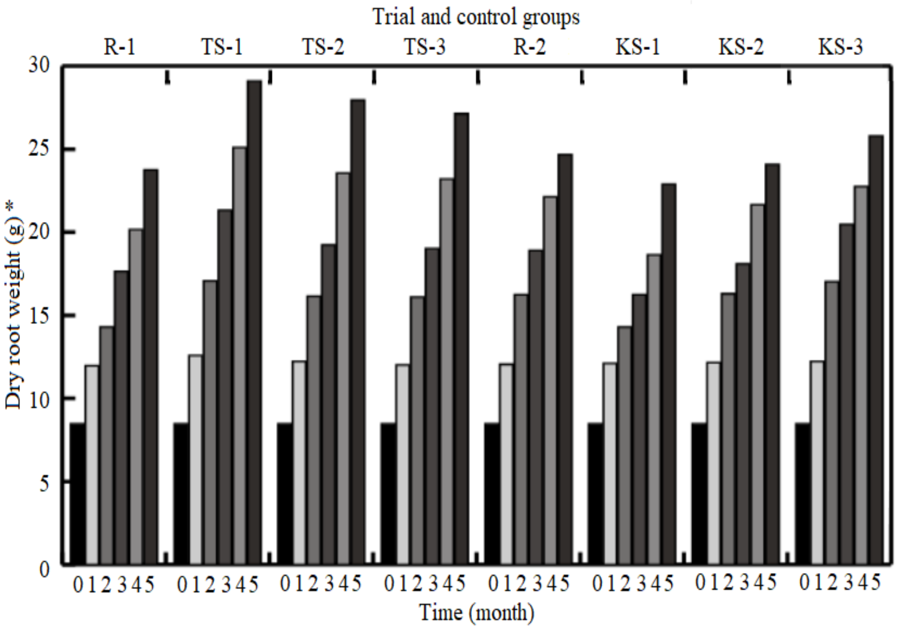

Figure 6- Monthly dry root weight chart of the trial and control groups (*, P≤0.05)

Figure 7- Root formation according to the type of medium and the amount of SAP

3.8. Calculation of labor costs

Irrigation and labor costs are presented in Table 4. When turf and SAP were used together, the average savings of 46% and the average savings of 51% were achieved in water usage and labor costs, respectively. When river sand and SAP were used together, there was an average reduction of 44% in water usage and an average reduction of 47% in labor costs. It was also stated by Nnadi (2012), Madakbaş et al (2014) and Bhagat et al (2016), that the irrigation of plants based on certain periods reduced labor costs and water usage by 30%-50% when SAP was used.

Table 4- Comparison of irrigation and labor costs

Code	*Labor Cost (TL)*	*Labor Cost-saving (%)*	*Water cost (TL)*	*Water-saving (%)*	*Total cost (TL)*
R-1	1.500	-	40.40	-	1.540.40
TS-1	900	40*	25.85	36*	964.65
TS-2	700	53*	21.00	48*	725.85
TS-3	600	60*	18.60	54*	621,00
R-2	2.500	-	64.65	-	2.518.60
KS-1	1.600	36*	42.85	34*	1.642.85
KS-2	1.300	48*	35.55	45*	1.335.55
KS-3	1.100	56*	30.70	52*	1.130.70

*, calculated by proportioning the data from the control group with the data from the trial group

4. Conclusions

This study based on the *Euonymus japonicus 'Aureomarginatus'* demonstrated the significance of the use of SAP within the cultivation medium in ensuring and maintaining balanced humidity. Depending on the amount of SAP used, the amount of water retained in the medium increased, along with the shoot formation ratio and root development. SAP was thus found to be usable in the cultivation of ornamental plants. Depending on the cultivation medium and the amount of SAP, SAP use resulted in an average decrease in water use of 45%, and an average decrease in labor costs of 49%.

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Effects of Herbal Vitamin D_3 and Phytase Supplementation to Broiler Feed on Performance, Bone Development and Serum Parameters of Broilers

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ABSTRACT
A trial was conducted to assess the effects of phytase supplementation and substitute Vitamin D_3 resource with Panbonis - a herbal vitamin D_3 source- (PAN) on performance, some carcass characteristics, tibia and serum parameters of broiler chickens. For this purpose, 11200 one-day-old, mixed sex (5600 male, 5600 female) Ross-308 chicks were administered 7 different diets based on corn, soybean and wheat throughout the 41-day trial. Dietary treatments; control group as T1 (5000 IU vitamin D_3), T2 (T1 + 500 FTU g^{-1} phytase), T3 (3000 IU vitamin D_3 + 500 FTU g^{-1} phytase + 100 mg kg^{-1} PAN) and T4 (3000 IU vitamin D_3 + 500 FTU g^{-1} phytase + 200 mg kg^{-1}PAN) were prepared to contain recommended levels of Ca-P however T5, T6 and T7 were formulated from T2, T3 and T4, respectively, by reducing 18% of Ca and P concentrations.

When overall results considered, there was no significant difference among treatments in terms of final live weight, mortality, weight gain, European Production Efficiency Factor (EPEF) and carcass parameters and mortality (P>0.05). While birds consuming diets containing phytase exhibited better FCR than control group without phytase (P<0.05), no additional improvement was obtained with PAN supplementation compared to other treatments without control group. Additionally partial replacement of PAN for synthetic form had no significant effect on tibia parameters and serum Ca, P levels even though serum Mg (in chicks fed sufficient Ca-P) and calcitriol were increased.

These results indicate that PAN could replace some part of synthetic vitamin D_3 without any adverse effect in broiler chickens. However, substitution rate of PAN in Ca and P deficient diets should be carefully studied more due to possible adverse effects on feed intake (12-41d) and weight gain (12-41d) as observed in the present study.

Keywords: Broiler, Calcium, Phosphorus, Vitamin D

1. Introduction

Vitamin D can derived from the diet or produced in the skin by means of sunlight. Dietary supplementation has become crucial due to intensive poultry farming not allowing substantial vitamin D synthesis. Vitamin D has been mostly provided to poultry by supplementation of the diet with synthetic forms of cholecalciferol (Vitamin D_3). Cholecalciferol must undergo change to form 25 hydroxycholecalciferol (25-OH-D_3) in liver and then in kidney 1,25- hydroxycholecalciferol (1,25-OH-D_3) which is active form of vitamin D_3. It has been long known that 1,25-OH-D_3 increases gut absorption of Ca-P therefore suboptimal vitamin D_3 levels in broiler diets adversely affect growth performance and bone development (Roberson & Edwards 1996; Biehl & Baker 1997). Besides, it was reported that enhanced phytate phosphorus availability through increasing gut mucosal phytase activity (Onyango et al 2006) thus decreased P excretion (Biehl & Baker 1997; Qian et al 1997) was obtained with supplementation

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of vitamin D_3 in broiler diets. Recently, there has been a growing interest in supplying vitamin D_3 with phytase in order to maximize utilization of phytate phosphorus. Some studies have evaluated combinations of phytase and the vitamin D analogs reported additive responses between those pair of additives. Snow et al (2004) obtained more bioavailable P release (0.07%) as a result of inclusion 1α-OH vitamin D_3 (5 µg) with phytase (300 IU) than addition of only 1α-OH vitamin D_3 (0.04%) or phytase (0.02%) in broilers fed 0.75% Ca and 0.13% none phytate phosphorus (NPP) diets. This additive effect was also observed in weight gain and tibia ash (mg tibia^{-1}) in the same research (Snow et al 2004). Besides, Biehl et al (1995) demonstrated that utilization of Zn and Mn responded additively in combination of vitamin D_3 analogues with phytase.

Stressful conditions, such as high bird density, heat stress, mycotoxicosis, enteritis, malabsorption syndromes and certain immune disorders may impair liver or kidney hydroxylation of cholecalciferol so directly adding active form of vitamin D_3 could be assumed to be advantageous for broilers. Dietary supplementation with synthetic 1,25-vitamin D_3 is not practical commercially because of the high cost of chemical synthesis. Thus, herbal vitamin D_3 sources have been discussed as possible alternative to the synthetic counterparts. The plant *Solanum glaucophyllum* was established that contains the active form of vitamin D_3 in a glycosidic bound form by researchers (Wasserman et al 1976; Boland 1988). Studies have indicated that inclusion of *Solanum glaucophyllum* as vitamin D_3 source in diets either led some improvements or at least had no adverse effect on growth performance and bone development of broilers and eggshell thickness of laying hens (Morris et al 1977; Cheng et al 2004; Bachmann et al 2013). With these benefits in mind, it may suggested that inclusion of *Solanum glaucophyllum* with phytase in broiler diets gives an opportunity to reduce dietary Ca-P levels. However, there is a lack of information about the effect of herbal source of vitamin D_3 (PAN), reduced phosphorus and calcium level besides phytase presence.

Therefore this study was conducted to test the effects of PAN with the presence of phytase at 2 levels of dietary Ca and P on growth performance, carcass characteristics, tibia and some serum parameters of broiler chickens.

2. Material and Methods

2.1. Birds and housing

The research was carried out in broiler research house of Beypilic Broiler Company, one of the largest broiler integration, in Bolu province, Turkey. 11200 one-day-old, mixed sex (5600 male, 5600 female) Ross-308 chicks were weighed and randomly allocated to 56 floor pens (6.5 × 2 m). Rearing conditions were set according to breeder guidelines (Anonymous 2014). Chicks were allowed *ad libitum* access to feed and water.

The research was conducted according to a completely randomized block design. Day old chicks were weighed and randomly distributed into 7 dietary treatments each has 8 replicates with 200 chicks in floor pens of experiment house through 41 days. Four phase feeding program was administered through the experiment as starter (0-11 days), grower (12-25 days), developer (26-35 days) and finisher (36-41 days).

2.2. Diets

All of the diets were formulated based on corn, soybean and wheat in pelleted form. Due to dietary additions of herbal vitamin D_3 were made at the expense of corn only compositions of diet 1, 2 and 5 were shown in Table 1. As could be seen in composition of diet 2 and 5, phytase addition to diets was made through considering Ca and P matrix values (Table 1). Diets for each feeding period were formulated to be isonitrogenous and isocaloric and to meet or exceed breeder guidelines (Anonymous 2014), but with different levels of available P (Pa), Ca and vitamin D_3 as given in Table 2. Starter feeds of all the treatments were produced as crumble form while grower and finisher feeds were pellet form in Beypilic Feed Mill.

Herbal Vitamin D_3 (Panbonis®-PAN): The herbal product contains 10 mg 1,25-Dihydroxycholecalciferol kg^{-1} in glycoside form and recommended to use 50-500 g per ton feed in addition to vitamin D from other sources. The product was supplied from Herbonis Animal Health, Basel, Switzerland.

Phytase: Phyzyme® XP (5000 FTU phytase g^{-1}) from Danisco Animal Nutrition, Marlborough, UK. 600 g phytase enzyme premix was included in 1 ton of diets to give 500 FTU g^{-1} which assumed to release 0.11% Ca and 0.12% available P.

2.3. Data collection

All the procedures of animal use and biological material collection were approved by the Ethics Committee of Animal Use under the protocol number 2014-18-137. All chicks were weighed at first, 11th and 41st days of the research. Feed Intake was recorded for 0-11 and 12-41 days. Mortality was recorded on a daily basis. FCR was calculated for 0-11 and 12-41 days. EPEF (European Production Efficiency) was also calculated at the end of the experiment. At 24th days of age 2 chicks from each replicates near to average weight of the pen were selected, identified and killed for bone and blood serum measurements. Blood samples (1-2 mL) were collected by heart puncture and placed in test tubes, centrifuged for 10 minutes at 3000 rpm, and the serum was then placed in duly identified Eppendorf tubes and immediately frozen until analyses for serum alkaline phosphatase (ALP), creatine kinase (CK), calcitriol (CTL), Mg, P and Ca. The left tibia from each bird was excised, sealed in plastic bags and stored at -20 ºC until further analysis. At the end of the trial 2 broilers from each pen was selected to assess carcass, drumsticks, breast meat yield. Carcass, drumsticks (with bones), and breast meat (with bones) were excised and weighed, then calculated as a percent of live body weight.

Table 1- Composition of basal diets; normal diet (T1), phytase applied diet with Ca and P matrixvalue (T2) and low Ca and P diet (T5) with phytase matrix applied, g kg^{-1} air dry

Ingredients	*Starter*			*Grower*			*Developer*			*Finisher*		
	T1	*T2*	*T5*	*T1*	*T2*	*T5*	*T1*	*T2*	*T5*	*T1*	*T2*	*T5*
Corn	473.43	482.86	496.42	508.46	517.86	527.19	546.71	556.11	563.47	505.02	515.30	521.76
SBM[1], 46.5% CP	225.65	224.28	228.49	153.54	152.15	155.30	128.96	127.56	128.08	163.26	167.64	162.38
Full fat soybean	150.26	149.95	141.61	158.55	158.28	152.17	135.24	134.98	132.70	163.71	155.76	161.20
Wheat	50.00	50.00	50.00	70.00	70.00	70.00	70.00	70.00	70.00	85.00	85.00	85.00
Poultry meal	25.00	25.00	25.00	45.00	45.00	45.00	50.00	50.00	50.00	-	-	-
Soy oil	14.00	11.00	8.00	22.00	19.00	17.00	30.00	27.00	25.00	35.00	33.00	30.00
CGM[2], 60% CP	20.00	20.00	20.00	8.00	8.00	8.00	8.00	8.00	8.00	12.00	12.00	12.00
DCP	19.95	13.90	8.85	16.09	10.03	6.38	14.60	8.54	4.89	17.68	11.64	7.97
$CaCO_3$	7.95	8.95	7.53	6.74	7.74	6.98	6.28	7.28	7.31	7.35	8.35	8.38
Choline Cl	0.37	0.36	0.36	0.28	0.28	0.28	0.25	0.25	0.25	0.36	0.36	0.35
Lysine HCl, 99%	3.37	3.24	3.29	2.61	2.49	2.53	1.99	1.87	1.90	1.50	1.38	1.40
DL-Methionine	3.32	3.16	3.15	2.53	2.37	2.37	1.97	1.81	1.80	2.42	2.27	2.26
Phytase	0.00	0.60	0.60	0.00	0.60	0.60	0.00	0.60	0.60	0.00	0.60	0.60
$NaHCO_3$	1.80	1.80	1.80	1.50	1.50	1.50	1.40	1.40	1.40	2.20	2.20	2.20
NaCl	2.30	2.30	2.30	2.10	2.10	2.10	2.10	2.10	2.10	2.50	2.50	2.50
Salinomycin	0.40	0.40	0.40	0.40	0.40	0.40	0.50	0.50	0.50	-	-	-
Nicarbacin	0.20	0.20	0.20	0.20	0.20	0.20	-	-	-	-	-	-
Mineral premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated												
Crude protein, %	23.00	23.00	23.00	21.00	21.00	21.00	19.60	19.60	19.60	19.40	19.40	19.40
ME, kcal kg^{-1}	3025	3025	3025	3150	3150	3150	3225	3225	3225	3225	3225	3225
Calcium, %	1.00	0.89	0.71	0.90	0.79	0.67	0.85	0.74	0.61	0.85	0.74	0.61
Total P, %	0.77	0.65	0.56	0.72	0.60	0.54	0.70	0.59	0.51	0.70	0.59	0.51
Available P, %	0.50	0.38	0.29	0.45	0.33	0.27	0.43	0.31	0.24	0.43	0.31	0.24
Met+Cys, %	1.07	1.07	1.07	0.95	0.95	0.95	0.86	0.86	0.86	0.86	0.86	0.86
Lysine, %	1.43	1.43	1.43	1.24	1.24	1.24	1.09	1.09	1.09	1.09	1.09	1.09
Analyzed												
Crude protein, %	23.15	23.05	22.95	20.96	20.98	21.15	19.40	19.45	19.62	19.55	19.45	19.32
ME, kcal kg^{-1}	3032	3018	3034	3165	3146	3142	3240	3227	3231	3219	3217	3209
Calcium, %	1.04	0.90	0.70	0.93	0.81	0.68	0.84	0.73	0.63	0.85	0.75	0.62
Phosphorus, %	0.79	0.68	0.58	0.73	0.62	0.55	0.71	0.60	0.51	0.72	0.60	0.52

[1], soybean meal; [2], corn gluten meal

Table 2- Experimental design

Treatments	*Phytase FTUg^{-1}*	*Herbal VitD$_3$, mg kg^{-1}*	*VitD$_3$ (IU)*	*Starter*		*Grower*		*Developer-Finisher*	
				Ca, %	*Pa^1%*	*Ca, %*	*Pa^1%*	*Ca, %*	*Pa^1%*
T1	0	0	5000	1.00	0.50	0.90	0.45	0.85	0.43
T2	500	0	5000	0.89 (1.00)	0.38 (0.50)	0.79 (0.90)	0.33 (0.45)	0.74 (0.85)	0.31 (0.43)
T3	500	100	3000	0.89 (1.00)	0.38 (0.50)	0.79 (0.90)	0.33 (0.45)	0.74 (0.85)	0.31 (0.43)
T4	500	200	3000	0.89 (1.00)	0.38 (0.50)	0.79 (0.90)	0.33 (0.45)	0.74 (0.85)	0.31 (0.43)
T5	500	0	5000	0.71 (0.82)	0.29 (0.41)	0.67 (0.78)	0.27 (0.39)	0.61 (0.72)	0.24 (0.36)
T6	500	100	3000	0.71 (0.82)	0.29 (0.41)	0.67 (0.78)	0.27 (0.39)	0.61 (0.72)	0.24 (0.36)
T7	500	200	3000	0.71 (0.82)	0.29 (0.41)	0.67 (0.78)	0.27 (0.39)	0.61 (0.72)	0.24 (0.36)

Parenthesis shows the levels after phytase matrix values applied as 0.11% Ca and 0.12% available P; [1], phospourus available

2.4. Chemical analysis

Moisture, ash, crude protein (Nx6.25), ether extract, crude fiber analyses of feed ingredients and proximate analysis, sugar and starch of diets were performed according to the methods of the Association of Official Analytical Chemists (AOAC 2005). The ME content of the diets were calculated according to the equation (ME [kcal kg^{-1}]= 53+38×[% CP+2.25×% ether extractable fat {EE}+1.1×% Starch+% Sugar]) developed by Carpenter & Clegg (1956). Before analysis, meat and fat were gently removed from tibia bones. The bones were dried overnight at 100 ºC, extracted in ether for 6 h, and burnt to ash in a muffle furnace at 600 ºC. The ash from each tibia was used for phosphorus analysis according to AOAC (2005). The serum samples were analyzed by using of test-kits of Roche Diagnostics for serum calcium, phosphate, magnesium, alkaline phosphatase and creatine kinase. Serum 1.25-dihydroxyvitamin D_3 (CTL) were analyzed by using ELISA-kits of Immunodiagnostic, Bensheim Germany.

2.5. Statistical analysis

The data were analyzed as a completely randomized block design with 7 dietary treatments and 8 replicates using the ANOVA procedure of the MINITAB (Statistical Software, version 13). All percentage data were subjected to arcsine square root transformation. When significant differences among groups were found, means were separated using the Tukey HSD test.

3. Results and Discussion

3.1 Performance Parameters, feed cost, some carcass characteristics and tibia parameters

The effects of treatments on performance at days of 0-11 and 12-41 were presented in Table 3 and whole period in Table 4. As shown in Table 3, performance parameters of 0-11d were not affected by treatments ($P>0.05$). However at days of 11-41, phytase supplementation significantly improved FCR compared with control. Also replacement of PAN at any level in low Ca-P level (T5, T6) significantly ($P<0.05$) decreased feed intake and level of 100 mg kg^{-1} replacement (T6) significantly decreased weight gain ($P<0.05$). As shown in Table 4 when whole growth period considered, there were no significant difference among treatments in terms of final live weight, weight gain, mortality and EPEF ($P>0.05$). On the other hand, phytase supplementation significantly improved FCR compared with control but, partial replacement of PAN for synthetic form had no improved effect on Feed Intake and FCR at any level.

The feed cost analysis for kg live weight given in table 4 showed that 100 mg kg^{-1} PAN supplementation (T6) into diets containing low Ca and P in the presence of phytase has significantly reduced the feed cost for kg live weight ($P<0.05$) compared to other treatments, except T7. Although cost benefits mainly created by reduced level of Ca and P in T5, T6 and T7, growth performance and FCR obtained by 100 mg kg^{-1} PAN supplementation had serious contribution in the feed cost reduction.

Table 3- Effects of herbal Vitamin D₃ and phytase supplementation on growth performance of broilers through 0-11 and 12-41 days

T*	*Initial weights, g*	*Live weight 11th day, g*	*Weight gain 0-11 days, g*	*FCR 0-11 days*	*Feed intake 0-11 days, g*	*Mortality 0-11 days*	*FCR 12-41 days*	*Feed intake 12-41 days, g*	*Weight gain 12-41 days, g*
1	43.51±0.17	309.52±2.04	266.01±2.06	1.24±0.02	328.68±4.44	4.56±0.71	1.73±0.08a	4014.51±54.78a	2321.57±28.00ab
2	43.59±0.13	313.11±2.59	269.52±2.63	1.21±0.01	325.61±2.57	4.03±0.69	1.71±0.09b	4031.31±39.09a	2364.22±17.07a
3	43.57±0.11	312.20±1.75	268.63±1.73	1.23±0.01	330.28±2.90	4.00±0.77	1.70±0.08b	3992.86±32.93a	2351.14±21.20a
4	43.53±0.11	312.82±3.34	269.28±3.34	1.24±0.02	332.35±3.00	4.25±0.82	1.69±0.07b	4018.98±36.80a	2377.09±27.91a
5	43.51±0.18	310.49±2.94	266.98±2.89	1.22±0.02	324.38±4.54	3.56±0.65	1.69±0.09b	3963.47±29.36a	2343.41±16.43ab
6	43.61±0.16	315.59±2.56	271.98±2.61	1.22±0.01	332.05±2.96	6.38±0.85	1.70±0.08b	3835.96±33.84b	2261.21±19.81c
7	43.54±0.12	306.68±2.47	263.13±2.38	1.25±0.01	328.98±3.04	4.88±0.52	1.69±0.06b	3863.32±43.53b	2284.37±24.73bc
P	0.991	0.119	0.121	0.590	0.498	0.230	0.004	0.005	0.005

a-c, means within a column with different superscripts are significantly different (P<0.05); *T, treatments

Table 4- Effects of herbal Vitamin D₃ and phytase supplementation on growth performance of broilers through 0-41 days and feed cost for kg live weight (FCFLW)

T*	*Final Live Weight,41th day,g*	*Weight Gain 0-41days*	*Feed Intake 0-41days*	*FCR 0-41days*	*Mortality 0-41days, %*	*EPEF*	*FCFLW, TL*
1	2731.11±24.57	2687.60±24.57	4506.79±43.80a	1.68±0.00a	10.81±0.78	340.31±3.59	2.69±0.03a
2	2729.20±15.77	2685.61±15.76	4436.91±28.14ab	1.65±0.00b	10.61±0.91	345.89±3.12	2.67±0.03ab
3	2729.13±9.54	2685.56±9.49	4428.08±21.97ab	1.65±0.00b	10.31±1.17	347.90±4.98	2.65±0.02ab
4	2742.09±25.19	2698.56±25.18	4432.82±31.70ab	1.64±0.00b	11.44±0.69	346.61±6.67	2.66±0.03ab
5	2711.68±29.59	2668.17±29.61	4378.11±41.88bc	1.64±0.00b	9.56±0.81	350.13±5.08	2.62±0.03ab
6	2727.67±23.38	2684.06±23.48	4413.60±37.81bc	1.64±0.00b	12.56±0.95	339.92±5.45	2.53±0.02c
7	2681.03±15.23	2637.49±15.28	4336.01±31.32c	1.64±0.00b	12.06±0.77	335.88±2.83	2.59±0.02bc
P	0.288	0.287	0,011	0.003	0.317	0.241	0.004

a-c, means within a column with different superscripts are significantly different (P<0.05); *T, treatments

The effects of treatments on some carcass characteristics were presented in Table 5 and tibia parameters in Table 6. As shown Table 5 carcass characteristics were not affected by treatments. As shown Table 6, phytase supplementation significantly decreased tibia P (% of dry-defatted tibia) and tibia ash (% of DM) compared with control (P<0.05) but partial replacement of PAN for synthetic form had no effect on tibia parameters.

Performance data showed that synthetic vitamin D_3 levels might be reduced by PAN addition without any adverse effect on weight gain, feed intake and FCR during the starter phase. However; from d 12-41, replacing 2000 IU synthetic vitamin D_3 with PAN in reduced Ca-P diets led to significantly decreased feed intake (P<0.05). In the same growth phase, it was also observed that chicks fed insufficient Ca-P needed 200 mg kg^{-1} PAN to maintain weight gain as 100 mg kg^{-1} PAN was adequate in broilers fed sufficiently in Ca-P. This result verifying other studies indicating that chicks needed more vitamin D_3 when suboptimal concentrations of Ca-P contents in diets (Baker et al 1998; Whitehead et al 2004; Rama Rao et al 2006). The present study in line with Cheng et al (2004) that reported no additional benefit in terms of FCR and weight gain by inclusion of *Solanum glaucophyllum* (dried leaves 7.5 g kg^{-1}) combination with phytase (1200 FTU kg^{-1}) in 0.59% Ca and 0.19% NPP containing diets. Addition to live weight, Bachmann et al (2013) found no significant (P>0.05) difference between product based on dried *Solanum glaucophyllum* leaves (providing 10 μg of 1,25- OH vitamin D_3) and free 1,25- OH vitamin D_3 (2.5 and 5 μg) in terms of tibia strength and stiffness in broiler chickens. Similarly, in the present study, tibia and carcass parameters of chicks fed diets that contain lower vitamin D_3 level (3000 IU kg^{-1} feed) with PAN were similar to that of the chicks fed diets contain regular vitamin D_3 level (5000 IU kg^{-1} feed) without PAN (P> 0.05). These results indicated that PAN could replace synthetic one in broiler diets without any adverse effect on tibia and carcass parameters. Phytase addition to diets including regular Ca-P levels did not significantly affect weight gain, FCR and mortality during the starter period (P>0.05). Chicks given the low Ca-P diets with phytase had similar weight gain and FCR when compared to birds consume regular Ca-P diets in the same period. This is in line with previous studies that indicate Ca-P level could be reduced by adding phytase without any depression in performance (Broz et al 1994; Sebastian et al 1996). Phytase addition improved FCR but feed intake and weight gain were depressed by reducing Ca-P level regardless of phytase or PAN supplementation during d 12-41 ($P<0.05$). Although tibia ash (% of DM) and tibia P (% of dry-defatted tibia) were negatively affected by decreasing levels of Ca and P in the diets, performance of broilers was not affected in general throughout study. Therefore, it could be claimed that Ca and P requirements of broilers needed to be carefully further studied, and re-evaluated.

Table 5- Effects of herbal Vitamin D_3 and phytase supplementation on some carcass characteristics % of live body weight

Treatments	*Carcass yield*	*Drumsticks*	*Breast meat*
1	71.78±0.44	31.59±0.27	32.58±0.38
2	71.89±0.45	32.03±0.29	32.08±0.37
3	72.13±0.35	32.00±0.20	32.31±0.36
4	71.87±0.34	31.36±0.25	32.81±0.36
5	72.67±0.64	32.18±0.31	32.46±0.45
6	72.28±0.35	31.57±0.30	32.86±0.34
7	71.63±0.54	31.65±0.31	32.21±0.44
P	0.733	0.321	0.727

Table 6- Effects of herbal Vitamin D_3 and phytase supplementation on tibia parameters

Treatments	*Dry-defatted tibia weight, g*	*Tibia ash, % of DM*	*Tibia P, % of ash*	*Tibia P, % of dry-defatted tibia*	*Tibia ratio, % in 24 day's live weight*
1	3.97±0.16	41.83±0.44a	17.13±0.17	7.18±0.12a	0.31±0.01
2	4.00±0.11	40.59±0.30b	16.94±0.14	6.89±0.09b	0.30±0.01
3	3.74±0.11	40.60±0.28b	16.97±0.09	6.90±0.06b	0.29±0.00
4	3.93±0.13	40.60±0.28b	17.03±0.07	6.92±0.07b	0.31±0.01
5	3.83±0.11	39.95±0.38bc	17.01±0.09	6.81±0.05b	0.30±0.00
6	3.60±0.11	39.77±0.25bc	17.05±0.08	6.80±0.06b	0.29±0.01
7	3.73±0.11	39.64±0.31c	16.98±0.06	6.74±0.05b	0.30±0.01
P	0.217	0.002	0.920	0.011	0.442

[a-c], means within a column with different superscripts are significantly different ($P<0.05$)

3.2. Serum parameters

The effects of treatments on some serum parameters were presented in Table 7. As shown in Table 7, while serum calcium, ALP and CK (except T2) concentrations were not significantly affected by treatments, serum CTL and Mg concentrations were increased with PAN replacement in broilers fed Ca-P adequately and P concentration was increased with phytase supplementation in broilers fed Ca-P adequately.

Table 7- Effects of herbal Vitamin D_3 and phytase supplementation on serum parameters

Treatments	*Calcium (mmol L^{-1})*	*Phosphate (mmol L^{-1})*	*Magnesium (mmol L^{-1})*	*ALP[1] (U l^{-1})*	*CK[2] (U l^{-1})*	*1,25-Dihydroxy Vit D_3 (pg mL^{-1})*
1	1.72±0.06	2.13±0.04d	0.73±0.04c	10.78±1.59	17.55±1.68b	118.81±6.82bc
2	1.79±0.08	2.34±0.04ab	0.74±0.05c	11.73±1.73	22.43±1.27a	113.50±4.83cd
3	1.74±0.08	2.40±0.06ab	0.95±0.05b	13.48±1.46	16.32±1.38b	125.89±6.53bc
4	1.70±0.06	2.45±0.06a	1.13±0.08a	8.05±1.07	17.24±1.74b	133.91±4.91ab
5	1.53±0.05	2.20±0.06cd	1.07±0.05ab	10.74±1.55	17.13±1.77b	142.45±6.56a
6	1.66±0.09	2.27±0.07bc	1.02±0.07ab	12.72±1.30	15.29±1.53b	98.74±4.86d
7	1.65±0.09	2.22±0.08cd	1.16±0.08a	9.94±1.56	17.87±1.80b	101.91±5.49d
P	0.243	0.001	0.000	0.114	0.051	0.000

[1], alkaline phosphatase; [2], creatine kinase; [a-d], means within a column with different superscripts are significantly different ($P<0.05$)

It has previously been demonstrated that the plant *Solanum glaucophyllum* contain vitamin D_3 as mainly derivatives of 1,25- OH-vitamin D_3 glycosidically-linked to carbohydrate units (Skliar et al 1992). Earlier studies evaluating the effects of *Solanum glaucophyllum* have found markedly increase in serum Ca (Uribe et al 1974) and 1,25-OH vitamin D_3 (Napoli et al 1977) concentration in rats, elevations of serum Ca and P levels in rabbits (Mautalen 1972). Similarly, in the present study, despite reduced synthetic vitamin D_3 levels in diet chicks were able maintain serum Ca and even tend to increase phosphate concentration by providing PAN. Moreover, enhancement of serum CTL and magnesium levels were detected in broilers fed sufficient Ca-P with PAN addition ($P<0.05$). This increase in serum Mg level could be thought as a result of increased intestinal phytase activity by providing PAN which was previously reported that synthetic vitamin D_3 derivatives as responsible (Biehl et al 1995; Onyango et al 2006). However, no significant increase observed in serum Ca and P levels in chicks fed PAN which may be hypothesized that Ca-P demand for bone tissue prevented significant increase of serum Ca-P concentrations in broiler chickens. In accordance with previous studies (Gray & Garthwaite 1985; Cheng et al 2004) reported that feeding with Ca-P deficient diets could stimulate renal production of CTL was observed when compared T2 and T5 in the present study. However, substitution of a particular proportion of PAN at the expense of synthetic source caused that decreased serum CTL levels ($P<0.01$) as serum Mg concentrations remained similar in birds fed lower Ca-P diets. The mechanism behind the decreased CTL levels with PAN is not clear but could be related lowered renal CTL synthesis by providing PAN. Moreover, it may be postulated that increasing metabolic consumption of CTL to maintain.

4. Conclusions

Considering the growth performance, feed cost for kg live weight, carcass, tibia and serum parameters it might be concluded that 100 mg kg^{-1} herbal source of vitamin D_3 (PAN) could be substituted for a portion of synthetic vitamin D_3 without any adverse effect and would have an economic contribution because of significant feed cost benefit in the presence of phytase at low Ca and P broiler diets. However, 200 mg kg^{-1} supplementation of PAN at low Ca and P diets must be carefully considered and further studied because of negative impact on feed intake and tibia ash found in the present study. The results of the present study were also showed that phytase supplementation of broiler diets could allow to reduce P and Ca level without any adverse effects on growth performance, carcass and serum parameters by giving economic benefits for feed cost.

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Efficacy of Native Entomopathogenic Nematodes on the Larvae of Tomato Leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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ABSTRACT
This study was conducted to determine the efficacy of native entomopathogenic nematodes (EPNs); *Steinernema affine* 46 (Bovien, 1937), *S. feltiae* 879 (Filipjev, 1934), *S. carpocapsae* 1133 (Weiser, 1955) and *Heterorhabditis bacteriophora* 1144 (Poinar, 1976) on the larvae of tomato leafminer *Tuta absoluta* (Meyrick). Bioassays were conducted in the laboratory at four different temperatures (10, 15, 20 and 25±1 ºC) in the plates and 30 infective juveniles (IJs) were inoculated to a single *T. absoluta* larva for each nematode species. After nematode inoculation, larvae were checked on the 3rd, 5th, 7th days and mortalities were recorded. All nematode species used in the study showed the lowest efficacy on the 3rd control day at 10 ºC and the highest efficacy on the 7th day at 25 ºC. *S. feltiae* 879 was found as the most efficient species with the highest mortality (91.67%) among EPNs used in the study. The results proved that *T. absoluta* larvae are highly susceptible to EPNs and the control of the pest by EPNs on this stage is successful.

Keywords: Tomato; *Tuta absoluta*; Larva; Biological control; Entomopathogenic nematodes

1. Introduction

Tomato (*Solanum lycopersicum*) is the most popular home garden and one of the most widely cultivated and important vegetables grown around the world both outdoors and in greenhouses for fresh consumption and processing due to its taste, color, flavor, and nutrient contents, but requires protection from a variety of pests.

The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is highly damaging to tomato plants causes nearly 100% yield losses in absence of control methods applied. *T. absoluta*, originated in South America, entered Europe (Spain) at the end of 2006. *Spread* rapidly in many other countries, was included in the EPPO A2 list due to its current distribution in the region (Roditakis et al 2010). In Turkey, *T. absoluta* was first recorded in 2009 in Urla (İzmir) and July-August of the same year it was also reported in Çanakkale. It caused significant crop losses in tomato production (Kılıç 2010; Kasap et al 2011).

Tuta absoluta has four main growth stages as egg, larvae, pupa and adult. The life cycle of the pest is completed 29-38 days with 10-12 generations per year based on environmental conditions (Vargas 1970; Urbaneja et al 2007). Females generally deposit their eggs on leaves, leaf veins; stem margins, sepals and green fruits. Its fecundity ranges from 60-120 eggs (Estay 2000). It has four larval instars and last instar larvae generally drop on the ground and pupate in soil on the leaves or other parts of the plants. Pupae complete their development in soil and adults

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emerge from soil after few days (Urbaneja et al 2007). Young larvae of *T. absoluta* start feeding on the plant by penetrating the tissues. They produce several mines in leaves, burrowing into stalks, apical buds, green and ripening fruits. These mines reduce the rate of photosynthesis and enable attacks by pathogens (Cáceres 1992).

Chemical control has been the first preferred method against *T. absoluta* since the pest was reported. However, successful chemical control of tomato leafminer is complicated due to its feeding behaviour because *T. absoluta* feeds internally within host mesophyll tissues. Chemicals may increase the cost of production but decrease the number of natural enemies of the pest (Desneux et al 2010). Also resistance to various insecticides has been occurred in different countries, adding further difficulties in the pest management (Siqueira et al 2000).

Alternatively, biological control may be considered using entomopathogenic nematodes (EPNs), due to their potential as biological control agents. EPNs are parasites of soil-borne organisms that infect pests occurring in, close or on top of the soil surface. In recent years, they have been used effectively to control many severe pests such as leafminers (Olthof & Broadbent 1990).

EPNs belong to the Steinernematidae and Heterorhabditidae families are identified by carrying symbiotic bacteria of the genus *Xenorhabdus* (Thomas & Poinar 1979) and *Photorhabdus* (Boemare Akhurst & Mourant 1993) in their intestine, respectively (Ehlers 1996; Boemare 2002). These specific bacteria have an important role in the pathogenicity; they multiply and kill the host insect by an induced septicemia. EPNs have many advantages such as their high reproductive potential, the ability to kill hosts quickly, high virulence, broad host range, easy mass rearing, and safety to plants, vertebrates, and other nontarget organisms (Kaya & Gaugler 1993). Due to these advantages of EPNs, many researchers from different countries conducted important studies on *T. absoluta*, which is a serious pest in tomato production, both under laboratory and field conditions to evaluate the potential of EPNs against this pest (Garcia-del-Pino et al 2013; Shamseldean et al 2014; Van Damme et al 2016; Ben Husin 2017; Kamali et al 2018).

We aimed at determining the efficacy of native EPNs on the larvae of *T. absoluta* in the laboratory. Unlike other studies, the efficacy of non-commercial native isolates on *T. absoluta,* obtained from different provinces of Turkey; *S. affine* 46, *S. feltiae* 879, *S. carpocapsae* 1133 and *H. bacteriophora* 1144 were determined at different temperatures in this study.

2. Material and Methods

2.1. Source and rearing of entomopathogenic nematodes

Steinernema affine 46, *S. feltiae* 879, *S. carpocapsae* 1133 and *H. bacteriophora* 1144, isolated from different provinces of Turkey in a previous project were reared at 25±1 ºC, 65±5% RH in the dark condition on the last instar larvae of greater wax moth *Galleria mellonella* L., (Lepidoptera: Pyralidae) (Bedding & Akhurst 1975; Kaya & Stock 1997). Nematode-infected *G. mellonella* larvae were put to White traps (White 1927). Freshly emerged IJs were harvested, rinsed in distilled water and stored at 8-10 ºC in tissue culture flasks within a week. Before being used for bioassays, the viability of EPNs was checked by a stereomicroscope.

2.2. Source and rearing of Tuta absoluta

Larvae, pupae and adults of *T. absoluta* were collected from different tomato production areas in Çanakkale and maintained in rearing cages (50x50x50 cm), covered with netting fabric for ventilation on tomato plants in a climate room at 25±1 ºC and 65±5% RH with a 16:8 h L:D photoperiod. Healthy larvae were collected for laboratory bioassays.

2.3. Laboratory bioassays

The efficacy of native nematode species against larvae of the tomato leafminer was evaluated in the laboratory. In each well of the 12 well plates one last instar larva of *T. absoluta* was placed and filled with moistened sterile sandy soil. The bioassays were conducted at 10, 15, 20 and 25±1 ºC with 30 IJs per each larva with two replicates. EPNs were applied on the soil surface, with only distilled water added to the control plates.

The larvae were checked on the 3rd, 5th and 7th days, counting the number of alive and dead larvae. Mortalities were recorded and infected larvae were transferred to White traps. Data were analyzed by one-way ANOVA followed by Duncan's multiple range test (P<0.05).

3. Results and Discussion

The findings of the study showed that the larvae of *T. absoluta* were susceptible to four EPN isolates used in this study. Different mortalities were obtained on the larvae based on the EPN species, temperatures and control days (Table 1-4). Among the species used in the study the most efficient species was found as *S. feltiae* 879 with 91.67% and the least efficient species was found as *S. affine* 46 with 83.33% (Table 1-3).

Table 1- Mortality of *Tuta absoluta* larvae caused by *Steinernema affine* 46 at four different temperatures and three control days Mean (%) ± SE

Temperature (ºC)	*3rd day*	*5th day*	*7th day*	*F value*
10	8.33±4.81 Aa	12.50±4.17 Aab	25.00±4.81 Ab	$F_{(3,15)}$= 3.55*
15	20.83±4.17 ABa	29.16±4.17 Bab	41.67±4.81 Bb	$F_{(3,15)}$= 5.70
20	37.50±4.17 Ba	45.83±4.17 Ca	66.66±0.00 Cb	$F_{(3,15)}$= 19.49
25	70.83±4.17 Ca	75.00±4.81 Dab	83.33±0.00 Db	$F_{(3,15)}$= 3.00
	$F_{(3,15)}$= 39**	$F_{(3,15)}$= 37.77	$F_{(3,15)}$= 58.00	

*, means in the row followed by the same capital letter for the EPN species are not significantly different (P<0.05); **, means in the column followed by the same small letter for the temperatures are not significantly different (P<0.05)

Table 2- Mortality of *Tuta absoluta* larvae caused by *Steinernema carpocapsae* 1133 at four different temperatures and three control days Mean (%) ± SE

Temperature (ºC)	*3rd day*	*5th day*	*7th day*	*F value*
10	12.50±7.98 Aa	12.50±4.17 Aa	25.00±4.81 Aa	$F_{(3,15)}$= 1.50*
15	20.83±4.17 ABa	29.16±4.17 Bab	41.67±4.81 Bb	$F_{(3,15)}$= 5.70
20	41.67±4.81 Ba	54.17±4.17 Cb	66.66±0.00 Cc	$F_{(3,15)}$= 11.57
25	62.50±4.17 Ca	75.00±4.81 Dab	87.50±4.17 Db	$F_{(3,15)}$= 8.10
	$F_{(3,15)}$= 16.53**	$F_{(3,15)}$= 40.24	$F_{(3,15)}$= 47.53	

*, means in the row followed by the same capital letter for the EPN species are not significantly different (P<0.05); **, means in the column followed by the same small letter for the temperatures are not significantly different (P<0.05)

Table 3- Mortality of *Tuta absoluta* larvae caused by *Steinernema feltiae* 879 at four different temperatures and three control days Mean (%)±SE

Temperature (ºC)	*3rd day*	*5th day*	*7th day*	*F value*
10	8.33±4.81 Aa	16.66±6.80 Aa	16.66±0.00 Aa	$F_{(3,15)}$= 1.0*
15	20.83±4.17 Ba	29.16±4.17 ABab	33.33±0.00 Bb	$F_{(3,15)}$= 3.5
20	50.00±0.00 Ca	54.17±4.17 Bab	70.83±4.17 Cb	$F_{(3,15)}$= 10.5
25	75.00±4.81 Da	83.33±0.00 Cab	91.67±4.81 Db	$F_{(3,15)}$= 4.5
	$F_{(3,15)}$= 56.28**	$F_{(3,15)}$= 42.87	$F_{(3,15)}$= 54.06	

*, means in the row followed by the same capital letter for the EPN species are not significantly different (P<0.05); **, means in the column followed by the same small letter for the temperatures are not significantly different (P<0.05)

In the current study high mortalities occurred on the larvae by high temperatures. The lowest efficacy was observed on the 3rd control day at 10 ºC and the highest efficacy was observed on the 7th control day at 25 ºC for all nematode species used in the study. *H. bacteriophora* 1144 caused no mortality on the larvae on the 3rd control day at 10 ºC and the efficacy started on the 5th control day at the same temperature. This is an expected situation as *H. bacteriophora* requires high temperatures for successful development and to infect and kill the host insect (Susurluk et al 2001; Susurluk & Ehlers 2008).

Table 4- Mortality of *Tuta absoluta* larvae caused by *Heterorhabditis bacteriophora* 1144 at four different temperatures and three control days Mean (%) ± SE

Temperature (ºC)	*3rd day*	*5th day*	*7th day*	*F value*
10	0.00±0.00 Aa	8.33±4.81 Aa	8.33±8.33 Aa	$F_{(3,15)}$= 0.75*
15	8.33±4.81 ABa	16.66±6.80 Aa	25.00±4.81 Ba	$F_{(3,15)}$= 2.25
20	33.33±0.00 Ba	41.67±4.81 Bab	50.00±0.00 Cb	$F_{(3,15)}$= 9.00
25	70.83±4.17 Ca	75.00±4.81 Cab	87.50±4.17 Db	$F_{(3,15)}$= 3.90
	$F_{(3,15)}$= 99.87**	$F_{(3,15)}$= 31.00	$F_{(3,15)}$= 43.10	

*, means in the row followed by the same capital letter for the EPN species are not significantly different (P<0.05); **, means in the column followed by the same small letter for the temperatures are not significantly different (P<0.05)

Similarly to our result, Van Damme et al (2016) reported that while *S. carpocapsae* and *H. bacteriophora* showed higher efficacy at 25 ºC (55.3 and 97.4% mortality) than at 18 ºC (12.5 and 34.2% mortality), *S. feltiae* caused 100% mortality at both temperatures. In the laboratory, *S. feltiae* and *S. carpocapsae* have high potential on tomato leaf miner larvae inside the mines of tomato leaves.

Among *Steinernema* species *S. affine* 46 showed the lowest efficacy on the larvae with 83.33 and followed by *S. carpocapsae* 1133 with 87.5 and *S. feltiae* 879 with 91.67%. *S. feltiae* and *S. carpocapsae* were successful species against various pests in many studies based on the EPNs efficacy (Lacey & Unruh 1998; Gözel & Güneş 2013; Gözel 2016).

In similar studies; (78.6-100%) (Batalla-Carrera et al 2010), 100, 52.3 and 96.7% (Garcia-del-Pino et al 2013), low pupal mortality (7%), caused by *S. feltiae* were reported (Türköz & Kaşkavalcı 2016). Also Shamseldean et al (2014) reported that *H. bacteriophora* caused higher mortality than *S. monticolum* both in the laboratory and field; 80-100%, 60-80%; 80-89%, 58-67% respectively against to *T. absoluta* larval stage using two different EPN applying dosages. Our results are consistent with the results of these studies.

In a study that was carried out in a tomato field, high efficacy was occurred with the same isolates on the larvae of *T. absoluta* by *S. feltiae* 879 90.7, 94.3%; *S. affine* 46 39.3, 43.7%; *S. carpocapsae* 1133 43.7, 49.3% and *H. bacteriophora* 1144 81, 83% in 2012 and 2013, respectively (Gözel & Kasap 2015).

4. Conclusions

This study focused on the potential of four native EPNs on the larvae of *T. absoluta* under laboratory conditions. *Tuta absoluta* is a devastating pest, difficult and expensive to control solely by chemicals; so IPM should be considered. EPNs are gaining attention in biocontrol researches day by day because they are highly pathogenic organisms and cause high mortality in a broad range of insects, so it is important to use these beneficial organisms in managing pests. In conclusion based on the literature knowledge and the results we obtained from this study EPNs are excellent candidates for biological control and have potential to control *T. absoluta*. All isolates tested in current study performed high efficacy on the larvae of *T. absoluta* particularly at high temperatures. Studies should be carried out with these species showing high efficacy in the laboratory against *T. absoluta* in IPM program as Kamali et al 2018 reported in their study.

It is necessary to carry out the conformity tests with EPNs and pesticides particularly the ones intensively used in tomato production to minimize the effects of these chemicals. Also, studies should be done using adjuvants compatible with EPNs by the most effective application method on the most effective application time against *T. absoluta* in the field, as EPNs encounter many different factors that affect their efficacy and survival adversely in foliar application. Furthermore, it could be useful to carry out studies in tomato fields by using the combinations of different adjuvants as SilwetL-77, used in Van Damme et al (2016), and Barricade® II, used in Ben Husin (2017), both in terms of less EPNs per area and high efficacy. Previous studies indicated that addition of adjuvants to nematode suspension increases EPNs efficacy by providing perfect conditions for nematode host finding and invasion, also increases nematode survival and reduces the time required by nematodes to enter a leaf, tissue, etc.

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Determination of the Hydraulic Properties of a Flat Type Drip Emitter using Computational Fluid Dynamics

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ABSTRACT
The objective of this study was to determine the hydraulic properties of a flat type emitter using Computational Fluid Dynamics with different turbulence models and model options. In addition, it is aimed to investigate the effects of the emitter hydraulic properties on the design when the same emitter is used in drip irrigation pipes with different wall thicknesses. The lowest mean percentage deviation between the measured flow rates and the calculated flow rates with turbulence models was found as 0.70% and 0.74% in the SST *k*-*ω* and Stress-Omega RSM turbulence model for the wall thickness of 0.25 mm pipe, respectively. Also, the mean percentage deviation for the laminar turbulence model was found to be -1.01%. The minimum MAE (0.021 L h^{-1}) and RMSE (0.028 L h^{-1}) values were found in the SST *k*-*ω* low-Re corr. turbulence model and the minimum MAPE (1.068%) was found in the laminar turbulence model.

Keywords: Turbulence models; Emitter design; CFD

1. Introduction

Drip emitters are used to dissipate pressure in the lateral line and discharge the water in drip irrigation system. This dissipation pressure in the emitter is generally carried out by labyrinth channels with narrow and long flow paths. The geometric structure (tortuous, orifice etc. flow path) and dimensions (channel with and depth, dentation angle, spacing, and height) of the labyrinth channels directly affect the hydraulic characteristics of the emitter. Decreasing the flow path dimensions makes it easier to reduce pressure dissipation, but increases the risk of clogging.

An important parameter in the emitter design is to determine the emitter flow rate depending on the operating pressure and the geometry of the emitter flow path, and the relationship between them can be described as follows (Von Bernuth & Solomon 1986).

$$q = kH^{x} \tag{1}$$

Where; *q*, emitter flow rate (L h^{-1}); *H*, emitter operating pressure (kPa); *k*, emitter flow coefficient (L h^{-1} kPa^{-x}); *x*, emitter flow exponent in dimensionless. The value of the coefficient of *k* in Equation 1 depends on the

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physical dimensions of the water passage paths in the emitter. The value of *x* characterizes the emitter's flow regime.

Conventionally the development process of a new emitter needs time-consuming and high-cost operations. Nowadays products can be designed by sophisticated visible models. It is also possible to perform performance analyzes such as kinematics, fluid dynamics, production processes in the 3D design in the computer. Thus, it is possible to obtain the products at a lower cost, with as possible higher performance and in a shorter time.

The flow regime for the geometry of water flow channels of a drip emitter can be defined by the dimensionless Reynolds number for a Newtonian fluid (Munson et al 2006).

$$Re = \frac{VD_h}{\nu} \quad (2)$$

Where; $V=q/A_{cs}$, average velocity of flow in the cross-sectional area in m s^{-1}; q, emitter flow rate in (L h^{-1}); $A_{cs}= w \cdot d$, cross-sectional area of emitter flow path (m^2); w and d, minimum width and depth of the emitter channels (m); $D_h=4wd/2(w+d)$, hydraulic diameter for a rectangular channel (m); ν, kinematic viscosity of fluid (m^2 s^{-1}) (Munson et al 2006; Wei et al 2006; Li et al 2006; Zhang et al 2016).

In the past studies, the Reynolds numbers were generally found between 100-1500 in the laminar flow region (Re <2000) due to the flow path of the emitter was relatively small and the flow rates were quite low. However, numerous researchers had indicated that the flow in this region was through the laminar to turbulence or turbulence due to the flow path in the emitter was tortuous and relatively small cross-section channels. Therefore, they stated that turbulence models would be more compatible for Computational Fluid Dynamics (CFD) applications (Wei et al 2006; Dazhuang et al 2007; Zhang et al 2007; Li et al 2008; Cicconi & Raffaeli 2009; Liu et al 2009; Philipova et al 2009; Wu et al 2013).

Palau-Salvador et al (2004) found 95% compatible results between the experimental and simulation values for emitters with labyrinth flow channel in CFD analysis. Wang et al (2006) stated that the difference between experimental and calculated results was less than 2% in terms of velocity and pressure distributions in emitter for standard k-ε turbulence model with standard wall function. Philipova et al (2009) and Wei et al (2006) showed that the pressure-flow relationships could be found with less than 4% error using the same turbulence model in labyrinth flow channels for the emitter.

In the CFD studies conducted on this subject, solutions were generally performed using selected a turbulence model. However, solution methods and mesh structure considered in the solution were not clearly indicated. Furthermore, there were no evaluations in the literature regarding the contribution of different turbulence models and wall applications to the solution. The objective of this study was to determine the hydraulic properties of a flat type emitter using CFD techniques with different turbulence models and model options, and presentation of the approach that gives the closest solution by comparing with experimental data. In addition, it is aimed to investigate the effects of the emitter hydraulic properties on the design when the same emitter is used in drip irrigation pipes with different wall thicknesses.

2. Material and Methods

2.1. Experimental studies

Drip irrigation pipes equipped with flat type drip emitters were used in this study. Nominal flow rate of selected drip emitter was 1.6 L h^{-1} at 100 kPa of operating pressure. The pipe wall thicknesses were 0.15 mm (6 mil), 0.20 mm (8 mil) and 0.25 mm (10 mil). The general view and dimensions of the tested drip emitter are shown in Figure 1.

The drip irrigation laterals were placed horizontally on the test stand presented in Figure 2 (Korukçu 1980; Mizyed & Kruse 1989). Water was supplied to the system passing through the disc filter by using a pump, and the operating pressures of 50, 80, 100, 120, 150, 200 and 250 kPa were adjusted by the control valves at the pump outlet. The pressure values were measured by a digital manometer (Keller LEO1, Switzerland) having a precision of <0.1% of the full scale. The flow rates of a total 30 of emitters in each drip irrigation pipes were measured using 1000 ml graduated cylinders at the different operating pressures (Bralts & Wu 1979; Mizyed & Kruse 1989).

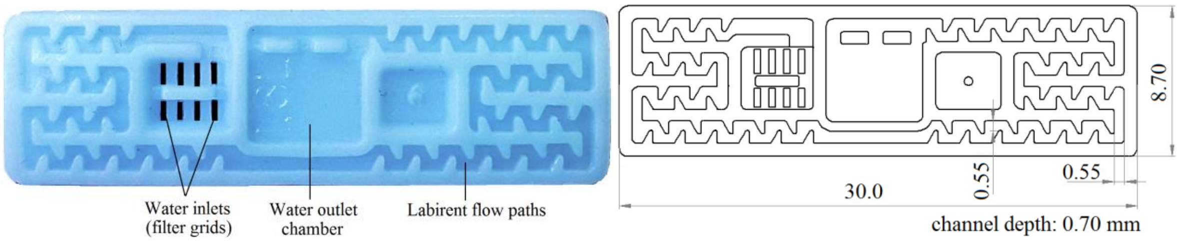


Figure 1- General view of the flat type integrated in-line drip emitter used in study

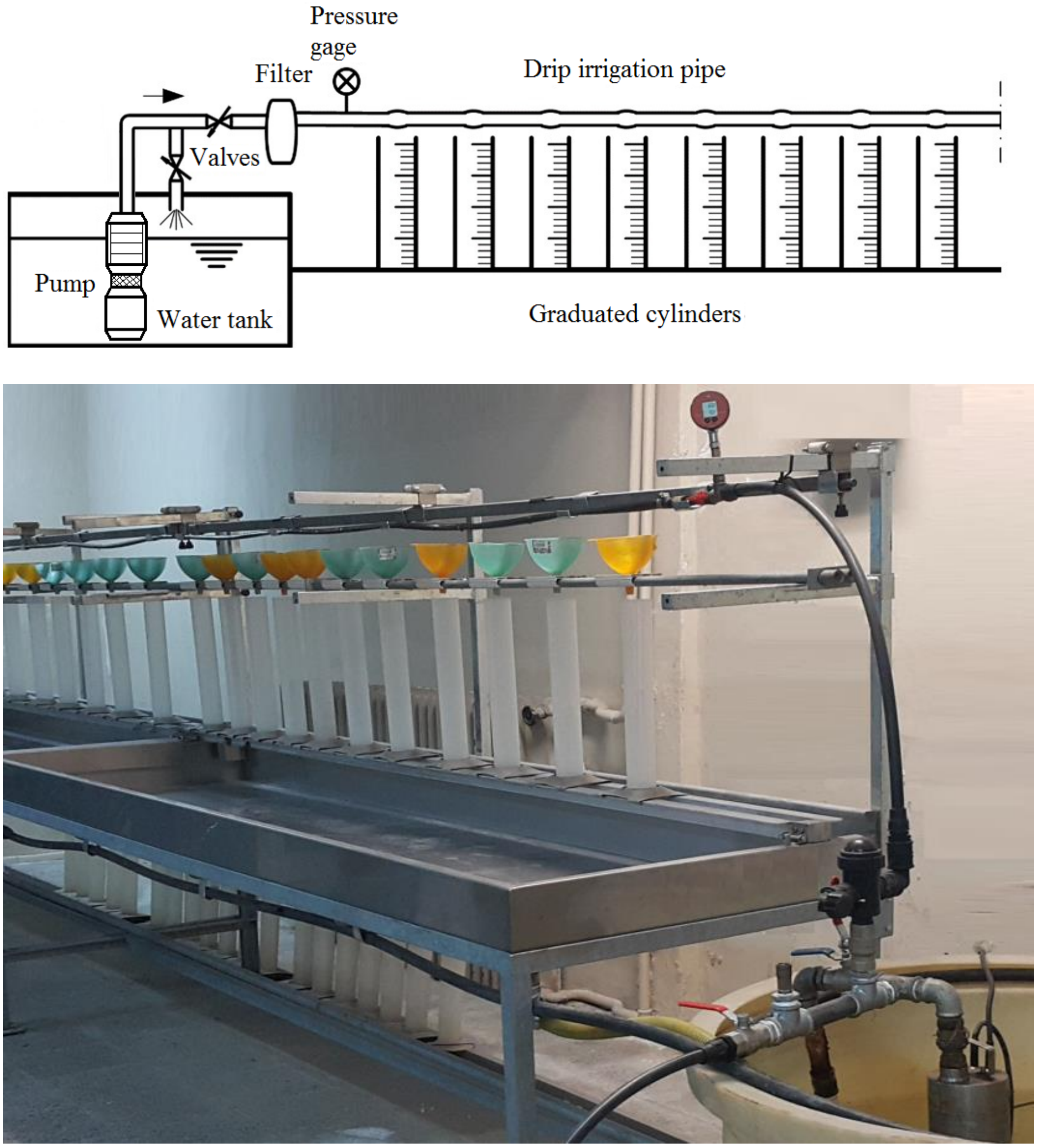


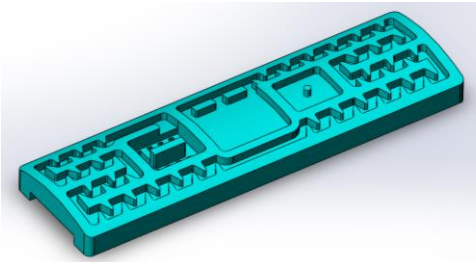
Figure 2. Schematics and experimental view of test stand

2.2. CFD analysis studies

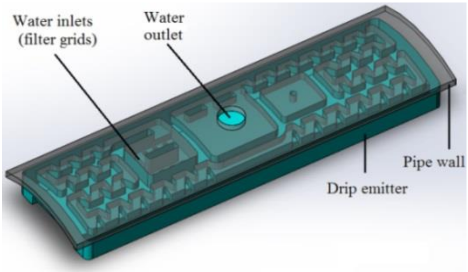
The flow analysis of emitter water flow channels were performed using commercial CFD software ANSYS Fluent 17.2 (ANSYS 2016).

2.2.1. Geometrical model and mesh generation

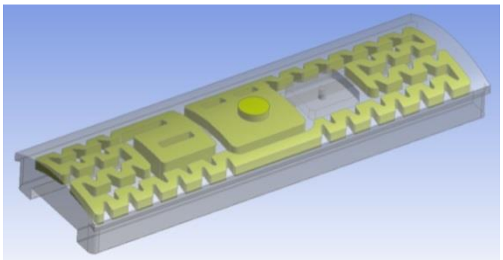
The geometrical models for drip emitter used in the study were created using ANSYS Design Modeler software. After created geometrical models, the mesh structures for water flow channels in drip emitter were formed using ANSYS Meshing software (Figure 3). The minimum dimension of one grid in mesh structure was selected as a 0.1 mm. The number of nodes and elements in this mesh structure were more than 3.5×10^5 and 2.2×10^5 respectively.



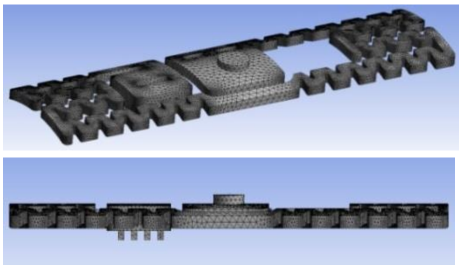
Drip emitter



Pipe wall and drip emitter



Water in flow channels in drip emitter



Mesh of the water in flow channels

Figure 3- Geometry of the drip emitter and mesh of the water flow channels

2.2.2. Mathematical model

The flow motion of the fluid can be described by the mass and momentum conservation equations. In the Newtonian, incompressible and steady-state flow condition, the density of fluid is the constant, and the conservation of mass or continuity equation is defined as:

$$\nabla \cdot \mathrm{v} = 0 \tag{3}$$

Similarly, an incompressible Newtonian fluid with constant viscosity, in vector notation of the Navier-Stokes equations is defined as:

$$\rho\left(\frac{\partial \mathrm{v}}{\partial t} + (\mathrm{v} \cdot \nabla)\mathrm{v}\right) = -\nabla p + \rho g + \mu \nabla^2 \mathrm{v} \tag{4}$$

In equations; ∇ is the vector operator ($\nabla = \partial/\partial x + \partial/\partial y + \partial/\partial z$); v, mean velocity vector (m s^{-1}); ρ, density of fluid (kg m^{-3}); p, static pressure (Pa); g, acceleration of gravity vector (m s^{-2}); μ, viscosity of fluid (Pa s) (Versteeg & Malalasekera 1995; Munson et al 2006; ANSYS 2016).

For the numerical analysis of Navier-Stokes equations in turbulence flow, the approach is called as Reynolds Averaged Navier Stokes (RANS) equations for the variation of fluctuating velocity, pressure, and other scalar quantities considering take the time-average. Various turbulence models are used in the RANS approach to analyzing the Reynolds stress tensor term appropriately, taking into account the effects of turbulence.

In this study, realizable k-ε turbulence model with enhanced wall treatment, SST k-ω turbulence model with low-Re corrections and production limiter options and Stress-Omega Reynolds Stress Model (RSM) with low-Re corrections and shear flow corrections options were considered for CFD analysis.

2.2.3. Boundary conditions and solution methods

In ANSYS Fluent analysis; the fluid was chosen as water, it was assumed to be a steady, incompressible, viscous and non-gravity effect. Inlet boundary condition was set to be operating pressures (50, 80, 100, 120, 150, 200 and 250 kPa) and outlet boundary condition was assumed to 0 kPa (atmospheric pressure). Hydraulic diameter of the flow channels was taken into account in Reynolds number calculations. Surface roughness height was not considered because of the quite low roughness height of the plastic producing material of the emitters. Simple algorithms and Second Order Discretization Schemes were used in all solutions. In the study, a limit value of 500 iterations was applied for the stability of the solution. The solution convergence accuracy was accepted to be 1×10^{-5}.

2.3. Statistical analysis

The mean absolute error (MAE), the root mean square error (RMSE) and the mean absolute percentage error (MAPE) were used to compare the differences between the experimental flow rate data and the predicted data using CFD models (Willmott & Matsuura 2005; Ding et al 2017). As it is known, the lowest values of these comparison criterion given below represent the highest model prediction.

$$\text{MAE} = \frac{1}{n}\sum_{i=1}^{n}\left|q_{i,Exp} - q_{i,CFD}\right| \tag{5}$$

$$\text{RMSE} = \left[\frac{1}{n}\sum_{i=1}^{n}(q_{i,CFD} - q_{i,Exp})^2\right]^{1/2} \tag{6}$$

$$\text{MAPE} = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{q_{i,Exp} - q_{i,CFD}}{q_{i,Exp}}\right| \tag{7}$$

Where; $q_{i,Exp}$ is experimental and $q_{i,CFD}$ is the simulation values, n is the number of data.

3. Results and Discussion

Drip emitter parameters and manufacturing variations depending on the measuring results for drip irrigation pipes with different wall thickness are given in Table 1. Also, calculated emitter flow rates depending on the inlet pressure using the CFD simulation models considered in the study, and the percentage deviation values according to the measured and calculated flow rates were shown in the same table.

The emitter flow exponent (x) values were found to be very close to 0.5 (Table 1). Therefore, it can be said that the emitter flow regime is fully turbulence (Von Bernuth & Solomon 1986). The emitter manufacturing variations values were found excellent according to the classification of ASAE standards for all wall thicknesses. It can be said that all emitter are manufactured quite close to each other in that production technology (Von Bernuth & Solomon 1986).

The emitter flow rate was found as 1.655 L h^{-1} for 0.25 mm pipe wall thickness at the nominal working pressure of 100 kPa for a considered emitter with the nominal flow rate of 1.6 L h^{-1}. These results indicated that the emitter was designed for a pipe wall thickness of 0.25 mm. It was determined that the emitter flow increased when the pipe wall thickness of the drip irrigation pipe decreased (Table 1). For example, it was found that the emitter flow rate increased by 3.2% and 5.14% at the nominal working pressure of 100 kPa for the pipes manufactured as 0.20 mm and 0.15 mm of pipe wall thickness instead of 0.25 mm. Cicconi & Raffaeli (2009) stated that the emitter flow rate in three different drip irrigation pipes increased from 4.7% to 12.2% when the pipe thickness decreased from 1.2 mm to 1.0 mm. The results were similar. This variation in flow rates may also be explained by the increase in emitter flow cross-sectional area due to the reduction of overlap of the emitter with the plastic material during the connection of the pipe and the drip emitter in the extruder line. In addition, it appears that the emitter flow cross-sectional area have more influence due to the increase in pressure on the material with less pipe wall thickness.

CFD simulation studies are generally carried out by assuming that the water flow path is constant and does not change by external factors. However, the experimental results showed that the change in pipe wall thickness had an effect on the flow rate due to the changes emitter flow path. Therefore, constant flow rates were found for each considered turbulence model. The comparison of the flow rates calculated with CFD and measured according to each pipe wall thickness is given as percentage deviation in Table 1. In addition, a comparison of the flow rates calculated with the turbulence models and measured at different working pressures for the pipe wall thickness of 0.25 mm are given in Figure 4.

Table 1- The results for drip emitter hydraulic properties and percentage deviation of drip emitter flow rates

Pipe wall thickness	*Emitter parameters*		*Manufacturing variations*	*Operating pressure*	*Experimental flow rate*	*CFD simulation models*							
	Flow coefficient	*Flow exponent*				*Laminar*		*SST k-ω low-Re corr.*		*Realizable k-ε enhanced wall*		*Stress-Omega RSM low-Re corr.*	
e	k	x	V_m^*	H	q_{exp}	*Predicted flow rate, q_{pre}*	*Percentage deviation***	*Predicted flow rate, q_{pre}*	*Percentage deviation*	*Predicted flow rate, q_{pre}*	*Percentage deviation*	*Predicted flow rate, q_{pre}*	*Percentage deviation*
(mm)	($L\ h^{-1}\ kPa^{-x}$)	-	-	(kPa)	($L\ h^{-1}$)	($L\ h^{-1}$)	(%)	($L\ h^{-1}$)	(%)	($L\ h^{-1}$)	(%)	($L\ h^{-1}$)	(%)
				50	1.150	1.150	0.00	1.127	2.00	1.163	-1.13	1.117	2.87
				80	1.483	1.481	0.13	1.438	3.03	1.512	-1.96	1.440	2.90
				100	1.655	1.656	-0.06	1.631	1.45	1.712	-3.44	1.614	2.48
0.25 mm (10 mil)	0.1555	0.5127	0.0191	120	1.798	1.815	-0.95	1.795	0.17	1.891	-5.17	1.791	0.39
				150	2.033	2.057	-1.18	2.031	0.10	2.135	-5.02	2.037	-0.20
				200	2.363	2.395	-1.35	2.364	-0.04	2.499	-5.76	2.394	-1.31
				250	2.628	2.724	-3.65	2.675	-1.79	2.822	-7.38	2.679	-1.94
				Avg.	-	-	-1.01	-	0.70	-	-4.27	-	0.74
				50	1.190	1.150	3.36	1.127	5.29	1.163	2.27	1.117	6.13
				80	1.525	1.481	2.89	1.438	5.70	1.512	0.85	1.440	5.57
				100	1.708	1.656	3.04	1.631	4.51	1.712	-0.23	1.614	5.50
0.20 mm (8 mil)	0.1594	0.5144	0.0239	120	1.870	1.815	2.94	1.795	4.01	1.891	-1.12	1.791	4.22
				150	2.085	2.057	1.34	2.031	2.59	2.135	-2.40	2.037	2.30
				200	2.440	2.395	1.84	2.364	3.11	2.499	-2.42	2.394	1.89
				250	2.730	2.724	0.22	2.675	2.01	2.822	-3.37	2.679	1.87
				Avg.	-	-	2.23	-	3.89	-	-0.92	-	3.93
				50	1.265	1.150	9.09	1.127	10.91	1.163	8.06	1.117	11.70
				80	1.605	1.481	7.73	1.438	10.40	1.512	5.79	1.440	10.28
				100	1.740	1.656	4.83	1.631	6.26	1.712	1.61	1.614	7.24
0.15 mm (6 mil)	0.1986	0.4734	0.0235	120	1.900	1.815	4.47	1.795	5.53	1.891	0.47	1.791	5.74
				150	2.115	2.057	2.74	2.031	3.97	2.135	-0.95	2.037	3.69
				200	2.465	2.395	2.84	2.364	4.10	2.499	-1.38	2.394	2.88
				250	-***	2.724	-	2.675	-	2.822	-	2.679	-
				Avg.	-	-	5.28	-	6.86	-	2.27	-	6.92

*, $V_m=S_q/q_{avg}$; S_q is the standard deviation; q_{avg} is the mean drip emitter flow rate; **, $100 \cdot (q_{exp}-q_{pre})/q_{exp}$; ***, the experimental data could not be measured because this pressure was higher than the strength of the pipe at this wall thickness

It was seen that the calculated flow rates with considered turbulence models except for realizable *k-ε* turbulence model with enhanced wall treatment were quite close to the measured flow rates for pipe wall thickness of 0.25 mm (Figure 4). Furthermore, as can be seen from Table 1, the lowest mean percentage deviation between the measured and the calculated flow rates was found as 0.70% in the SST *k-ω* turbulence model for the pipe wall thickness of 0.25 mm. Moreover, another close mean percentage deviation was found as

0.74% with the Stress-Omega RSM. The mean percentage deviation for the laminar turbulence model, which is accepted as a model with an easy and fast solution, was found to be -1.01%. This results found in the study were less than the percentage deviations of 2 to 5% found by various researchers in simulation studies (Palau-Salvador et al 2004; Wang et al 2006; Wei et al 2006; Zhang et al 2007; Cicconi & Raffaeli 2009; Philipova et al 2009; Wu et al 2013). Cicconi & Raffaeli (2009) stated that there were differences from 8.5 to 12.8% in the flow rates for the experimental and the *k-ε* turbulence models depending on pipe wall thickness and emitter flow channel depth for the flat drip at 100 kPa inlet pressure. These results clearly show that the emitter should be designed considering the pipe wall thickness.

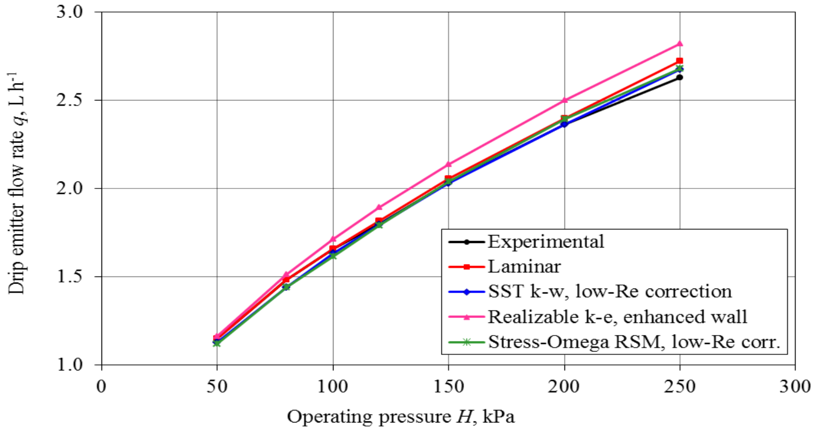


Figure 4- Comparison of the operating pressure and flow rate relationship of the drip emitter for experimental and CFD models for 0.25 mm pipe wall thickness

The MAE, the RMSE and the MAPE were calculated to compare the performances of considered CFD models for 0.25 mm pipe wall thickness and the results were given in Table 2. In addition to the comparison criterion, to show the harmony between the experimental and predicted flow rates for four CFD turbulence models is given in Figure 5.

Table 2- The MAE, RMSE and MAPE results for all simulation models for pipe wall thickness of 0.25 mm

CFD Models	*Mean absolute error MAE (L h^{-1})*	*Root mean square error RMSE (L h^{-1})*	*Mean absolute percentage error MAPE (%)*
Laminar	0.025	0.040	1.068
SST *k-ω* low-Re corr.	0.021	0.028	1.224
Realizable *k-ε* enhanced wall	0.089	0.107	4.281
Stress-Omega RSM low-Re corr.	0.030	0.034	1.723

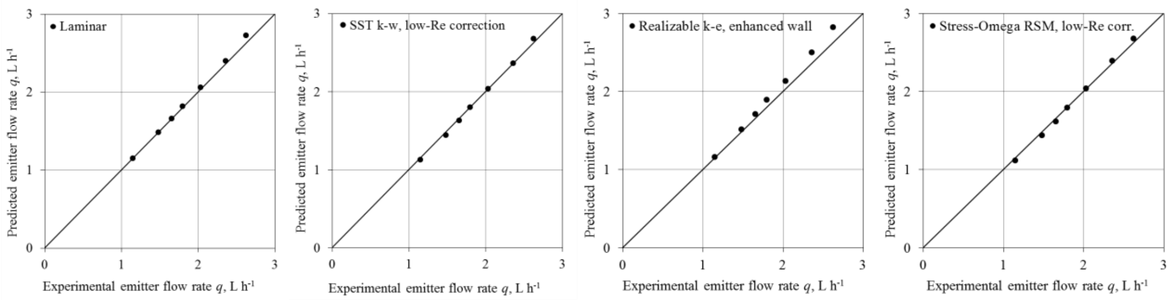


Figure 5- Comparison of the experimental and predicted drip emitter flow rate for the considered turbulence models

As seen in Table 2, among those CFD simulation models, the minimum MAE (0.021 L h^{-1}) and RMSE (0.028 L h^{-1}) values were found in the SST *k-ω* low-Re corr. turbulence model and the minimum MAPE (1.068%) was found in the laminar turbulence model. These results indicated that the flow rate could be calculated with the SST *k-ω* low-Re correction and the laminar turbulence models with a very low error compared to the measured emitter flow rate, and the emitter design could be done quickly and easily using simulation method (Figure 5).

The flow characteristics of the emitter flow channels were also investigated in the study. For this purpose, the flow coefficient (*k*) and the flow exponent (*x*) were calculated with the considered CFD simulation models. The results were also compared with the experimental data for the pipe wall thickness of 0.25 mm (Table 3). In addition, the flow velocities and the Reynolds numbers at the lowest and highest emitter flow rates in the minimum flow cross-section area of the emitter were determined. These results are given in Table 3.

Table 3- Comparison of the emitter properties, and range of flow velocity, Reynolds numbers for experimental data and different CFD simulation models for pipe wall thickness of 0.25 mm

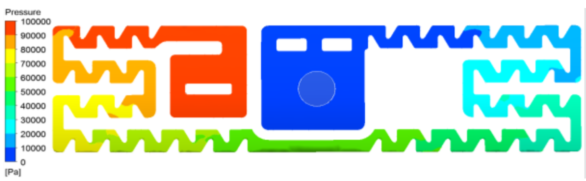
Data source	*Emitter parameters*		*Range of flow velocity*	*Range of Reynolds number*
	Flow coefficient	*Flow exponent*		
	k (L h^{-1} kPa^{-x})	*x*	*V (m s^{-1})*	*R_e*
Experimental	0.1555	0.5127	0.83-1.90	506-1156
Laminar	0.1426	0.5329	0.83-1.97	505-1198
SST *k-ω*, low-Re correction	0.1366	0.5384	0.81-1.93	495-1177
Realizable *k-ε*, enhanced wall	0.1355	0.5503	0.84-2.04	512-1242
Stress-Omega RSM, low-Re corr.	0.1306	0.5477	0.81-1.93	492-1179

When the k and x values, which denote the emitter dimensions and flow properties, were analyzed, it was found that the results of the laminar and SST *k-ω* Low-Re corr. models were the closest to the experimental values. The Reynold numbers were calculated depending on the flow rate of the emitter, and found between 500 and 1250, approximately. These values were found in harmonious with the other researches (Li et al 2006; Wei et al 2006; Dazhuang et al 2007; Zhang et al 2007; Zhang et al 2016).

The flow velocity distributions at the different input velocities and the pressure distributions for laminar and SST *k-ω* Low-Re corr. models were examined and the results are given in Figure 6. There was no significant difference observed between the two models in terms of pressure and flow velocity distributions. It was observed that the pressure might be reduced to the desired value in the flow channels.

Laminar model

SST *k-ω* Low-Re corr. model



Pressure distributiuon

Flow velocity distribution

Figure 6- Pressure and flow velocity distributions in emitter flow channels

When flow velocity distributions were analyzed, it was seen that high flow velocities such as 3 m s^{-1} occurred depending on the cross-section in the flow channel. The flow velocity decreased considerably on the walls of the flow channels, and generally, 0.5 m s^{-1} and higher flow velocities occurred in the center of the channel. In the flow channels, it was seen that the flow velocity at the bottom of the sharp corners in the flow direction was very low (less than 0.5 m s^{-1}), and especially in these regions vortices appeared (Figure 7). It can be said that small particles entering the drip emitter may accumulate in these regions over time due to low flow velocities and vortices in these regions may cause clogging in the emitter (Patil et al 2013). As a result, it can be said that these negative effects may be prevented by designing these zones with a slightly rounded rather than sharp edges of the water flow path.

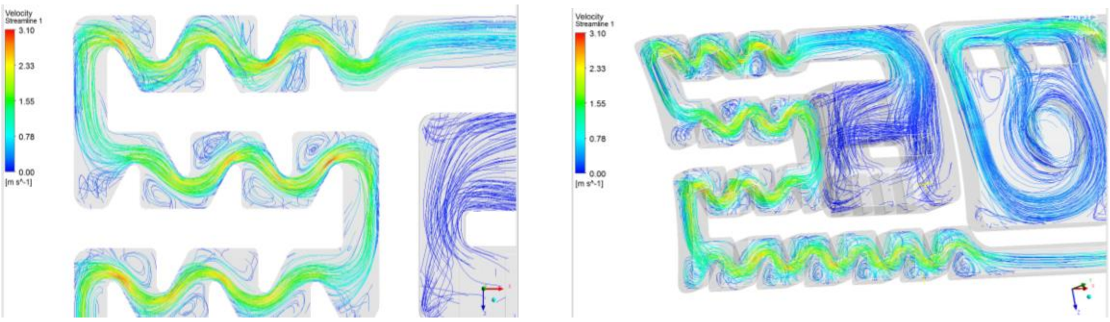


Figure 7- The details of the velocity distributions in emitter flow channels for SST *k-ω* model

4. Conclusions

The following points were concluded from the conducted study:

- The emitter flow rate was found as 1.655 L h^{-1} for 0.25 mm pipe wall thickness at the nominal working pressure of 100 kPa for a considered emitter with the nominal flow rate of 1.6 L h^{-1}.

- The lowest mean percentage deviation between the measured flow rates and the calculated flow rates with CFD turbulence models was found as 0.70% and 0.74% in the SST *k-ω* and Stress-Omega RSM turbulence model for the wall thickness of 0.25 mm pipe, respectively. Also, the mean percentage deviation for the laminar turbulence model was found to be -1.01%.

- The minimum MAE (0.021 L h^{-1}) and RMSE (0.028 L h^{-1}) values were found in the SST *k-ω* low-Re corr. turbulence model and the minimum MAPE (1.068%) was found in the laminar turbulence model.

It is clear from the results that, the wall thickness of the drip irrigation pipe is an important parameter on emitter flow rate. Because of this reason, this parameter should be considered in the simulation studies carried out on emitter design. In addition, the differences between the solution approaches of the CFD simulation models affect the prediction of the drip emitter flow rates. As can be seen from the results, the emitter flow rate could be predicted very closely to the experimental data with the proper choice of the wall function and the well-configured mesh structure. Thus, it is possible to realize designing of an emitter with using simulation methods introduced in the study, with less effort, lower cost, minimum mold revision and in a short time.

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Mite Diversity (Acari) from Ornamental Plants in Erzurum in Turkey

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ABSTRACT
Mite species belongs to Tetraychychidae (3); Tenupalpidae (2), Stigmaeidae (1), Tydeiidae (2), (Acari: Prostigmata); Phytoseiidae (10) (Acari: Mesostigmata) and Acaridae (1) (Astigmata) were identified on woody ornamental plants and shrubs in Erzurum (Eastern part of Turkey). The samples were collected from Erzurum (Centrum, Pasinler, Köprüköy, Horasan, Aziziye-Ilıca, Aşkale, Tortum, Uzundere and Çat districts) with a weekly interval between April to October during 2015 and 2016. Five species were phytophagous mites (belong to Tenuipalpidae and Tetranychidae), while the others are considered as predators or feed on microorganisms, neutral in their habitats. Nineteen mite species representing in three orders: *Amblyseius andersoni* (Chant), *Kampimodromus aberrans* (Oudemans), *Euseius finlandicus* (Oudemans), *Typlodromus cotoneastri* (Wainstein), *Neoseiulus astutus* (Beglyarov), *Phytoseius finitimus* Ribaga, *Typhlodromus* (*Anthoseius*) *kerkirae* Swirski and Ragusa, *Typhlodromus* (*Anthoseius*) *recki* (Wainstein), *Paraseiulus soleiger* (Ribaga), *Neoseiulella tiliarum* (Oudemans), *Zetzellia mali* (Ewing), *Tydeus kochi* Oudemans, *Tydeus californicus* (Banks), *Tetranychus urticae* Koch, *Bryobia rubrioculus* (Scheuten), *Bryobia praetiosa* Koch, *Cenopalpus pulcher* (Canestini & Fanzago) *Brevipalpus californicus* (Banks), *Tyrophagus putrescentiae*. These results showed that Erzurum has rich biodiversity especially concerning predatory mite fauna. *T. urticae* (Schrank), was the most abundant and common phytophagous species (53.11%) while some other species were represented only one specimen (*Neoseiulus astutus* (Beglyarov) (Phytoseiidae). Most preferred hosts plants were *Philadelphus coronarius* L. (Hydrangeaceae) (8), *Malus coronaria* L. (Rosaceae) (7) and *Rosa canina* L. (Rosaceae) (6) while *Syringa vulgaris* L. (Oleaceae), *Salix sp.* (Salicaceae) and *Rosa pisiformis* (Christ) (Rosaceae) were populated by only (2) and (1) mite species respectively.

Keywords: Acari; Erzurum; Ornamental plants; Phytoseiidae; Tetranychidae

1. Introduction

Ecological condition in Erzurum and its neighbourhoods area are not suitable for several exotic outdoor ornamental plants to grow. However, the area is located phytogeographically in ancient Mesopotamian area. Therefore, plant species which can grow naturally in these areas can provide a rich ecological diversity. Erzurum is located at 2000 m altitude. Ornamental plants have several functional and aesthetic landscape values by being a native tree and small tree species of the region (Irmak 2013).

Several surveys were conducted to determine the mite species associated with woody ornamentals and shrubs of World-wide. It was reported several new mite species for the Hungary (Ripka et al 2002; 2005; Szabó et al 2009). In Turkey, some mites especially belong to Tetranychidae, Tenuipalpidae, Eriophyidae and Phytoseiidae

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species were identified on ornamental and park plants (Alaoğlu 1991; 1996; Faraji et al 2011; Çobanoğlu et al 2016).

There are limited numbers of the study exists on agricultural and park areas in Erzurum. Some plant-parasitic and phytoseiid species were reported in Erzurum and Erzincan previously. *Bryobia rubrioculus* Scheuten, *Tetranychus urticae* Koch, *Typhlodromus kazachstanicus* Wainstein (Ecevit 1981); *Euseius finlandicus* (Oudemans), *Kampimodromus aberrans* Oudemans, *Paraseiulus soleiger* (Ribaga), *Paraseiulus talbii* (Athias-Henriot), *Phytoseius echinus* Wainstein & Arutunjan, *Neoseiulella tiliarum* (Oudemans) and *Typhlodromus* (*Anthoseius*) *rhenanus* (Oudemans) (Alaoğlu 1996). Beside this *Neoseiulus zwoelferi* (Dosse) and *Proprioseiopsis okanagensis* (Chant) species were reported in that region (Çobanoğlu 1989).

Therefore, it is a major necessitates a thorough investigation into the mites associated with ornamental plants for determination of the mite biodiversity in Erzurum. Survey studies can provide detection of predatory species are rich which are potential for biological control of economical important pests on ornamental plants.

The goal of the study is to determine mite species on shrubs and woody ornamental plants in Erzurum plateau during 2015-2016.

2. Material and Methods

The surveys were carried out on woody ornamentals and shrubs in Erzurum. The samples were collected 11 different host plants: 1. *Rosa canina* L. (Rosaceae), 2. *Rosa dumalis* Bechst. (Rosaceae), 3. *Rosa pisiformis* (Christ) (Rosaceae), 4. *Ribes aureum* Pursh. (Grossulariaceae), 5. *Philadelphia coronarius* L. (Hydrangeaceae), 6. *Robinia pseudoacacia* L. (Fabaceae), 7. *Hippophae salicifolia* Robert (Elaeagnaceae), 8. *Syringa vulgaris* L. (Oleaceae), 9. *Malus coronaria* L. (Rosaceae), 10. *Malus floribunda* L. (Rosaceae), 11. *Salix* sp. with 9 different districts of Erzurum (Centrum, Pasinler, Köprüköy, Horasan, Aziziye (Ilıca), Aşkale, Tortum, Uzundere, and Çat), which is located Eastern part of Turkey, during 2015 and 2016 (Figure 1; Table 1).

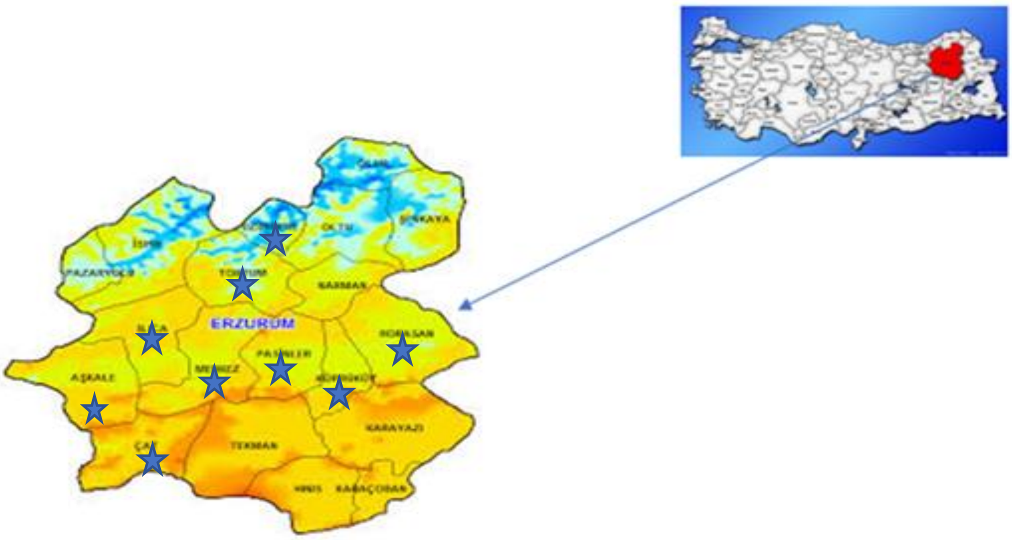

Figure 1. Sampling localities: Erzurum (Eastern Part of Turkey) (★)

The samples were deposited in the mite collection at Ankara University and Atatürk University Plant Protection Department of Turkey. The samples were collected from April to October of each year (2015 and 2016) with a weekly interval. The mites were collected randomly from the different height of the plants. The sampling was conducted from ornamental plants in landscape areas such as parks, gardens, roadsides, school and home gardens.

All the mite samples were extracted by Berlese funnel. The mites were kept in 70% ethanol and afterwards, cleared in Lacto-phenol solution and prepared in Hoyer's medium, later they dried for 15-20 days at 50 °C (Henderson 2001).

The collections were made by K. Akçakoyunluoğlu (Atatürk University).

All the identification of the samples were made by S. Çobanoğlu, according to; Jeppson et al (1975), Kolodochka (1978), Papadoulis et al (2009), Faraji et al (2011), Seeman & Beard (2011) and Çobanoğlu et al (2016). World distribution and host range are considered, according to by Migeon & Dorkeld (2006-2016); Moraes et al (2004) and Demite et al (2015). GPS data of the collection sites are shown in Table (1).

Table 1- Coordinates of the sampling localities

Location	*GPS*
Atatürk University Campus	N 39º 53′ 59. 1″, 041º 14′ 19. 0″ E 1880 m
Pasinler	40° 02'56.0" N, 41° 35'21.7" E 1660 m
Uzundere	40° 32'52.7" N, 41° 34'23.6" E, 1089 m
Çat	39° 35'42.4" N, 40° 57'59.6" E, 1918 m
Aziziye	39° 55'09.3" N, 41° 12'20.7" E, 1800 m
Aziziye-Dadaşkent	39° 55′ 09.3″ N, 041°12′ 20.7″ E, 1806 m
Tortum	40° 18'35" N, 41° 31'33" E, 1637 m
Tortum Chelles area,	40° 33′ 55.6″ N, 41° 35′ 46.2″ E, 1009 m
Aşkale	39°56'03.1" N, 40° 43' 32. 8" E, 1662 m
Aşkale-Çayköy	39º 56′44. 2″ N, 040º 48′ 18. 5′ E, 1720 m
Abdurrahman Gazi Forest	39° 52′ 36. 0″ N, 41° 18′ 35. 2″E, 2170 m
Erzurum centrum,	39° 48'59.7" N, 41° 04'32.8″ E, 1880 m
Palandöken	39° 48′ 41. 2″ N, 041° 07′ 10. 0″ E, 1990 m

3. Results and Discussion

In total, 241 specimens were collected from shrubs and woody ornamental plants which were 98 from *Rosa canina* L. (Rosaceae), 67 from *R. aureum* and 39 from *P. coronarius*.

In a total of 19 mite species were identified in 3 different orders and 6 families. Three species of Tetranychidae, two Tenuipalpidae, ten Phytoseiidae and one species from Astigmata were identified. In these Tetranychidae and Tenuipalpidae species are plant-parasitic while one species of Acaridae is saprophagous and the rest are predatory species (Phytoseidae and Stigmaeidae) while Tydeidae includes neutral species (Table 2, 3).

Table 2- List of identified mite species in Erzurum-Turkey

Order	*Family*	*Mite species*
Mesostigmata	Phytoseiidae	*Amblyseius andersoni* (Chant) *Kampimodromus aberrans* (Oudemans) *Euseius finlandicus* (Oudemans) *Typlodromus cotoneastri* (Wainstein) *Neoseiulus astutus* (Beglyarov) *Phytoseius finitimus* Ribaga *Typhlodromus* (*Anthoseius*) *kerkirae* Swirski and Ragusa *Typhlodromus* (*Anthoseius*) *recki* (Wainstein) *Paraseiulus soleiger* (Ribaga) *Neoseiulella tiliarum* (Oudemans)
Prostigmata	Stigmaeidae	*Zetzellia mali (*Ewing)
	Tydeidae	*Tydeus kochi* Oudemans *Tydeus californicus* (Banks)
	Tetranychidae	*Tetranychus urticae* Koch *Bryobia rubrioculus* (Scheuten) *Bryobia praetiosa* Koch
	Teuipalpidae	*Cenopalpus pulcher* (Canestini & Fanzago) *Brevipalpus californicus* (Banks)
Astigmata	Acaridae	*Tyrophagus putrescentiae* (Schrank)

3.1. Phytoseiidae berlese

During surveys, 10 Phytoseiidae species were identified.

Amblyseius andersoni (Chant) 1957

Material examined - Pasinler, 11.VII.2015 (2♂♂) (*Rosa dumalis subsp. bossieri*). Uzundere, 12.VII.2015 (2♀♀, 1♂); 15.08.2015 (2♂♂); 05.IX.2015 (5♀♀, 3♂♂) (*P. coronarius*); Tortum, 15.VIII.2015 (1♂) (*R. aureum*); (1♀) (*R. dumalis*).

Comments: *Amblyseius andersoni* is a common predatory mite species in Turkey*;* It was reported on *Citrus* spp. *Convolvulus* sp., *Corylus*, *Fagus, Fragaria, Juglans*, *Lycopersicum, Malus*, *Populus*, *Prunus*, *Pyracantha, Rubus, Sambucus*, *Solanum* and *Tilia* sp. from Ankara, Adana, Adapazarı, Antalya, Bartın, Bolu, Bursa, Edirne, Giresun, Hatay, Istanbul, Kırklareli, Rize, Sakarya, Tekirdağ, Tokat and Trabzon (Faraji et al 2011; Kumral & Çobanoğlu 2015). *A. andersoni* was collected in Erzurum as 16 specimen 7.05% (Table 3).

Table 3- Mite species and host plants range*

Family	*Mite species*	*Host plants*											*Number of specimens*	*Ratio (%)*
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*		
Tetranychidae	*T. urticae*	+	+	+	+	+	+	-	-	+	+	-	128	53.11
	B. rubrioculus	-	-	-	-	+	-	-	-	+	-	-	3	1.24
	B. praetiosa	-	+	-	-	-	-	-	-	-	-	-	1	0.41
Phytoseiidae	*A. andersoni*	-	+	-	+	+	-	-	-	-	-	-	17	7.05
	N. astutus	-	-	-	-	-	-	+	-	-	-	-	1	0.41
	T. cotoneastri	-	-	-	+	-	-	-	-	-	-	-	1	0.41
	T. (A.) kerkirae	+	-	-	-	+	-	-	-	-	-	-	5	2.07
	T(A.). recki	-	-	-	-	-	-	+	-	-	-	-	1	0.41
	P. finitimus	-	-	-	+	-	-	-	-	+	-	-	5	2.07
	E. finlandicus	+	+	-	+	+	+	-	+	+	-	-	45	18.67
	K. aberrans	-	-	-	-	-	-	-	-	+	+	-	9	3.73
	P. soleiger	-	-	-	-	-	-	-	-	-	-	+	2	0.82
Tydeidae	*N. tiliarum*	+	-	-	-	+	-	-	-	+	-	-	4	1.65
	T. kochi	-	-	-	-	-	+	-	+	-	-	-	2	0.82
	T. californicus	-	+	-	-	-	-	-	-	+	-	+	11	4.56
Tenupalpidae	*B. californicus*	-	-	-	-	+	-	-	-	-	-	-	1	0.41
	C. pulcher	-	-	-	+	+	-	-	-	-	+	-	3	1.25
Stigmaeidae	*Z. mali*	+	-	-	-	-	-	-	-	-	-	-	1	0.41
Acaridae	*T. putrescentia*	+	-	-	-	-	-	-	-	-	-	-	1	0.41
In total													241	100.00

*: 1. *Rosa canina L.*	*4.Ribes aureum*	*7.Hippophae salicifolia*
2.Rosa dumalis	*5.Philadelphia coronaria*	*8.Syringa vulgaris*
3.Rosa pisiformis	*6.Robinia pseudoacacia*	*9-Malus coronaria*
10. Malus floribunda	*11. Salix* sp.	

Euseius finlandicus (Oudemans 1915)

Material examined **-** Atatürk University, 02.VII.2015 (10 ♂♂) (*P. coronarius*); Aşkale, 11.VI.2015 (3♀♀, 5♂♂) (*R. dumalis*); Pasinler, 11.VII.2015 (3♂♂) (*R. aureum*), *S. vulgaris* L., *M. hybrida*; Uzundere, 05.IX.2015 (2♂♂) (*R. pseudoacacia*) (*R. canina*); Aziziye, (1♀, 2♂♂) 16.VIII.2015. (*R. canina*); Aziziye-Dadaşkent, 14.VIII.2015 (1♂) (*R. dumalis*); Tortum Falls, 12.VII.2015 (5♀♀, 8 ♂♂) (*M.communis*); Tortum, 12.VII.2015 (5♂♂) (*R. aureum*).

Comments**:** *Euseius finlandicus* is common on different plants throughout Turkey (Faraji et al 2011). It was recorded from eggplants, tomatoes, pepper, nightshade plants and *Datura stramonium* L. (Rosaceae) from Ankara, Bursa and Yalova Region of Turkey (Çobanoğlu & Kumral 2014; 2016; Kumral & Çobanoğlu 2016). It was identified from Erzurum (Alaoğlu 1996). It is abundant in Erzurum on parks and ornamental plant at 18.67%.

Kampimodromus aberrans (Oudemans 1930)

Material examined - Uzundere, 07.VI.2015 (1♀) (*M. cronoria*); Tortum Falls, 07.VI.2015; 12.VIII.2015 (8♀♀) (*M. floribunda*).

Comments: *Kampimodromus aberrans* is abundant on various plants, throughout Turkey. It was mentioned from different orchards, park plants, woody ornamentals and shrubs in Turkey (Alaoğlu 1996; Faraji et al 2011). It was collected in Erzurum at 3.73% (Table 3).

Neoseiulus astutus (Beglyarov 1960)

Material examined - Aşkale-Çayköy, 05.IX.2015, (1♀) (*H. salicifolia)*

Comments: *Neoseiulus astutus* was recorded in Ankara on *Salix babylonica* L. (Salicaceae) by Çobanoğlu (2002). It is a rare species and presented by only one specimen.

Paraseiulus soleiger (Ribaga1904)

Material examined - Aşkale-Çayköy, 06.VI.2016 (2 ♀) *(Salix sp.)*

Comments: *P. soleiger* was found on *M. communis*, stone fruits; *Prunus avium* L., *Prunus. persica* L., *Prunus domestica* L. (Rosaceae), *Ulmus* sp. and *Vitis vinifera* L. (Vitaceae), from Adana, Ankara, Amasya, Erzincan, Erzurum, Gümüşhane, Isparta, Istanbul, Kastamonu, Manisa, Nevşehir, Niğde, Tekirdağ, Tokat and Van Lake Basin (Alaoğlu 1996; Faraji et al 2011). It was represented only two specimens.

Phytoseius finitimus Ribaga (1904)

Material examined - Aziziye- Dadaşkent, (2♀♀) (R. *aureum*) Tortum, 05.IX.2015 (2♀♀); Tortum Falls, 05.IX.2015 (1♀♀) (*M. oronaria* (L).

Comments: *P. finitimus* is a very common species throughout Turkey. This species was reported on *Ailanthus* sp., *Citrus* spp., *Clematis vitalba*, *Cornus mas*, *Corylus avellana*, *Cydonia vulgaris*, *Ficus carica*, *Malus communis*, *Morus nigra*, *Prunus communis*, *Prunus domestica*, *Prunus spinosa*, *Rhamnus* sp., *Ribes* sp., *Rosa* sp., *Solanum melongena*, *Rubus* sp., *Ulmus campestris*, *Ulmus* sp., *Vitis vinifera* (Çobanoğlu & Kumral 2014; Faraji et al 2011). It is collected at 2.07% (Table 3).

Typlodromus cotoneastri Wainstein (1961)

Material examined - Tortum, 05.IX.2015, (1♀♀) (*R. aureum*).

Comments: *T. cotoneastri* was reported on Betulaceae, Cornaceae, Rosaceae, Fagaceae, Moraceae, Pinaceaea, Ulmaceae, Caprifoliaceae and Vitaceae. It is distributed Ankara, Antalya, Bitlis, Edirne, Erzincan, Karabük, Kırklareli, Tekirdağ (Faraji et al 2011). It is collected in Erzurum at 0.41%.

Typhlodromus (*Anthoseius*) *kerkirae* Swirski & Ragusa (1976)

Material examined - Uzundere- Centrum, 05.IX.2015 (5♀) (*R.canina*).

Comments: It was collected on *Quercus* sp in Adana (Döker et al 2016). It s collected at 2.07% in Erzurum.

Typhlodromus (Anthoseius) recki Wainstein (1958).

Material examined - Aşkale-Çayköy, (1♀) (*H. salicifolia*).

Comments: This species was collected from *Citrus* spp., *Clematis vitalba* L. (Ranunculaceae), *M. communis, Pinus nigra* J. F. Arnold, *Pyrus elaeagnifolia Kotschyana* (Rosaceae), *Ribes* sp., *R. canina., V. vinifera* in Adapazarı, Amasya, Ankara, , Burdur, Bursa, Edirne, Gümüşhane, İçel, Isparta, Istanbul, Izmir, Kars, Kastamonu, Konya, Muğla, Nevşehir, Niğde, Tekirdağ, Tokat, Zonguldak (Faraji et al 2011). It is collected as one specimen.

Neoseiulella tiliarum (Oudemans 1930).

Material examined - Aşkale-Centrum, 11.VII.2015 .(2♂♂) (*R. canina*), Tortum Falls, 12.VII.2015 (1♀) (*M. cronoria*); Uzundere-centrum, 05.IX.2015 (1♂) (*P. coronarius*).

Comments: It was reported*; C. avellana, Crataegus* sp., *Juglans regia* L. (Juglandaceae), *M. communis, Prunus cerasus* L. (Rosaceae), *P. domestica*, from; Ankara, Amasya, Burdur, Bursa, Edirne, Erzincan, Erzurum, Gümüşhane, Isparta, Istanbul, Kastamonu, Konya, Nevşehir, Niğde, Tekirdağ, Tokat, Yalova (Alaoğlu 1996; Faraji et al 2011). It is collected at 1.65%.

3.2. Family tenuipalpidae berlese

Cenopalpus pulcher Pritchard & Baker (1958)

Material examined– Aziziye-Dadaşkent; 29.VI.2015 (1♀) (*R. aureum*); 05.IX.2015 (1♀) (*P. coronarius*); Tortum falls, 12.VIII.2015 (1♀) (*M. floribunda*).

Comments: Tenuipapids are plant parasitic species and known as Flat Mites. It was reported from Ankara, Bursa, Istanbul, Izmir, Konya Niğde, Samsun and Tokat from pome and stone fruits (Çobanoğlu et al 2016). It is presented at 1.25%.

Brevipalpus californicus (Banks 1904)

Material examined - Uzundere, 05.IX.2015 (1♀) (*P. coronarius*).

Comments: This species was collected on Citrus trees in Mersin and from Aegean vineyards (Göven et al 1999). *B.* californicus is rare species and collected as only one specimen.

3.3. Family tetranychidae donnadieu

Tetranychus urticae Koch (1836)

Material examined - Atatürk Univ. Campus, 27.V.2015 (1♀) (*R. aureum*), 05.VII.2015 (38 ♀♀) (*R. canina*). Pasinler, 06.VI.2015 (1♀), 11.VI.2015 (1♀), 16.VIII.2015 (4♀♀) (*R. aureum*), (1♀) (*M. hybrida*); 16.VIII.2015 (6♀♀, 1♂) (*R. pisiformis*). Uzundere, 07.VI.2015 (1♂) (*R. canina*); 12.VII.2015 (1♀) (*R. pseudoacacia*); 12.VII.2015 (9♀♀) (*P. coronarius*)*;* 12.VII.2015 (1♀ 1♂) (*R. dumalis*); 05.IX.2015 (2♀♀) (*R. canina*), 05.IX.2015(9♀♀) (*P. coronarius*); (1♀) (*R. pseudoacacia*). Çat, 11.VII.2015 (3♀, 8♂♂) (*R. canina*). Aziziye, 11.VI.2015 (6♀♀) (*R. aureum*). Aziziye-Dadaşkent, 14.VIII.2015 (1♀); 15.VIII.2015 (2♀♀) (*R. aureum*), Tortum, 12.VII.2015 (1♂) (*R. canina*); 12.VII.2015 (3♀♀) (*R. aureum*); 15.VIII.2015 (1♀) (*M. communis*). 05.IX.2015 (1♀)(*R. pisiformis*); 05.IX.2015 (3♀♀)(*R. aureum*).Tortum Falls area, 15.VIII.2015 (1♀) (*M. communis*). Aşkale, 16.VIII.2015, (2♀♀) (*R. dumalis*); 16.VIII.2015 (5♀♀, 1♂) (*R. aureum*). Abdurrahman Gazi Forest, 30.VIII.2015, (1♀), (*R. aureum);* Erzurum centrum, 30.VIII.2015 (1♀) (*P. coronarius*); (5♀♀, 3♂♂) (*R. pisiformis)*; Palandöken, 30.VIII.2015 (2♀♀) (*R. dumalis*).

Comments: *T. urticae* is an important plant parasitic mite species which has distributed world-wide with more than 150 host plants and distributed all around Turkey (Ecevit 1981; Çobanoğlu & Kumral 2014; Kumral & Çobanoğlu 2015; 2016). It is very common on the ornamental plants in Erzurum. It was found 53.11% of the identified samples. It was collected from eight host plant species in Erzurum (Table 3).

Bryobia rubrioculus (Scheuten 1857)

Material examined - University Campus-Erzurum, 27.V.2015 (2♀♀) (*M. hybrida*); Uzundere- Centrum, 05.IX.2015 (1♀) (*P. coronarius*).

Comments: *Bryobia* is belongs of Bryobiinae and present several important species worldwide (Migeon & Dorkeld 2006-2016). It is a serious pests on apple, apricot, plum, each and walnut trees. It was reported on *Corylus avellana, citrus, Cotoneaster horizontalis* Decne. (Rosaceae), *Lonicera tatarica* L. (Caprifoliaceae), *Mahonia aquifolium* (Pursh) (Berberidaceae), *M. floribunda, Prunus cerasus, Thuja orientalis* L. (Cupressaceae), *V. vinifera* from, Adana, Amasya, Ankara, Denizli, Çanakkale, Erzurum, Izmir, Manisa, Niğde Van and Black Sea region (Ecevit 1981; Göven et al 1999; Uysal et al 2001; Özman & Çobanoğlu 2001). *Bryobia* species collected mostly on neglected trees. It was found (1.24%) among the other mite species on *P. coronoria* and *M. coronoria* (Table 3).

Bryobia praetiosa Koch (1836)

Material examined - Aziziye-Dadaşkent; 29.VI.2015, (1♀) *R. dumalis*.

Comments: This species was reported Ankara on *Fragaria ananassa*, *M.communis and Ficus carica* (Uysal et al 2001). It was found 0.41% in Erzurum.

3.4. Family stigmaeidae oudemans

Zetzellia mali Ewing (1917)

Material examined - Aşkale-Centrum, 11.VII.2015 (1♀) (*Rosa canina*)

Comments: Zetzellia *mali* is important predacious mite, it was reported from Ankara, Bilecik, Bursa, Samsun Van and Tokat (Doğan 2007; Çobanoğlu & Kumral 2014; Kumral & Çobanoğlu 2015). It is not very common in Erzurum and identified on *R. canina* as 0.41% (Table 3)

3.5. Family tydeidae kramer

Tydeus californicus (Banks 1904)

Material examined − Aşkale-Çayköy, 06.VI.2015 (11♀♀) (*R. dumalis, M. cronoria*, *Salix* sp.)

Comments: This is very small soft bodied mites and mostly feed on mites eggs. *Tydeus californicus* is a cosmopolitan species. It is also accepted as neutral species. *T. californicus* reported on hazelnut, pome and stone fruit trees all around Turkey (Çobanoğlu & Kaźmierski 1999). It was represented by 4.56 % in this region (Table 3)

Tydeus kochi Oudemans (1928)

Material examined− Pasinler, 11.VII.2015, (1♀), (*S.vulgaris*); Uzundere, 05.IX.2015, (1♀) (*R. pseudoacacia*).

Comments: *Tydeus kochi* is a common species and reported on *Fragaria ananassa* Duchesne (Rosaceae) from Aydın (Çobanoğlu & Kaźmierski 1999). We identified 0.82% among the other mites (Table 3).

3.6. Family acaridae

Tyrophagus putrescentiae (Schrank 1781)

Material examined− Atatürk University Campus, 05.VII.2017 (1♀) (*R. canina*)

Comments: *Tyrophagus putrescentiae* prefer mostly stored products and common species throughout Turkey in different habitats and saprohagous mites. It was determined fresh onion fields in Izmir (Kılıç et al 2012), *Sinapis arvensis* L. (Brassicaceae) in Çanakkale (Kasap et al 2013), *Lycopersicon esculentum*, *Solanum dulcamara* L. and *Solanum nigrum* L. (Solanaceae) in Ankara, Bursa, Yalova (Çobanoğlu & Kumral 2014; Kumral & Çobanoğlu 2015). It represented by only one specimen.

4. Conclusions

Nineteen mite species are identified on woody ornamental plants in Erzurum. *T. urticae*, *B. rubrioculus*, *T. kazachstanicus*; *E. finlandicus*, *K. aberrans*, *P. soleiger*, *P. talbii* (Athias-Henriot), *P. echinus*, *N. tiliarum*, *T.* (*A.*) *rhenanus*, *N. zwoelferi* and *P. okanagensis* were identified previously (Ecevit 1981; Çobanoğlu 1989; Alaoğlu 1996). The rest of identified species in the parks and ornamental plants in Erzurum (*A. andersoni*, *T. cotoneastri*, *N. astutus*, *P. finitimus*, *T.* (*A.*) *kerkirae*, *T.* (*A.*) *recki*, *Z. mali*, *T. kochi*, *T. californicus*, *B. praetiosa*, *C. pulcher*, *B. californicus* and *T. putres*centiae) are new records for this region. Erzurum has rich mite diversity especially concerning of beneficial mites because of rich host plants diversity and protected environment. Phytoseiidae members play important role as potential for biological control of economically important plant parasitic pests. Therefore, it is important to protect them in the environment. It will be useful to do more extensive studies in that area of Turkey.

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Eggshell Water Vapor Conductance and Shell Structural Characteristics of Broiler Breeder in Different Flock Ages

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ABSTRACT
The aim of the study was to investigate eggshell traits; including eggshell water vapor conductance, eggshell thickness, pore density, egg weight loss as well as eggshell surface area and volume in 28 and 40 week-old broiler breeders. Furthermore, the phenotypic correlations among the eggshell characteristics were determined. The eggs were ranged from 54.41 g to 60.78 g and 54.71 g to 60.62 g in 28 and 40 wk-old age groups, respectively. All eggs were weighed and numbered before setting and were monitored individually until the end of the incubation. Mean eggshell water vapor conductance (G) value was 11.00±1.01 mg H_2O day^{-1} $torr^{-1}$ in 28 wk-old flock and 11.99±1.28 mg H_2O day^{-1} $torr^{-1}$ in 40 wk-old flock respectively (P<0.05). The mean value of shell thickness and pore density mean along with the two regions (broad end and equator) were higher and significant in 28 wk-old flock (P<0.01). Egg weight loss was not found to be significant. Surface area was higher in 40 wk-old flock while as volume was higher in 28 wk-old flocks (P<0.01). There was no correlation between eggshell thickness and pore density. Both eggshell thickness and pore density were having significant positive correlation along with three eggshell regions in each flock ages (P<0.01).

Keywords: Water vapor conductance; Eggshell thickness; Pore density; Correlation

1. Introduction

Water vapor conductance is one of the important factors that influence the embryonic gas exchange and development of the embryo (Hamidu et al 2007). Moreover, it determines the respiratory pattern of the egg that is influenced by eggshell properties including the number of pores and thickness of eggshell (Peebles & Brake 1987). During embryonic growth, the gas exchange of the avian embryo is limited by the diffusion of gases through the pores of the eggshell. However, few studies have carried out on the influence of genetic selection of eggshell characteristics, like eggshell conductance (Tullett 1990). It has been observed that eggs from older hens have higher eggshell water vapor conductance, this variation could have arisen from the difference of the breeder age (Roque & Soares 1994). Therefore, to make an ideal gas exchange during the embryonic development, the conductance of eggshell should be considered as a functional tool besides other factors like a gaseous composition, barometric pressure of the surrounding natural air, the hatchery ventilation rate, humidity and embryonic oxygen uptake (Visschedijk 1991).

Changes in shell porosity have been seen among flocks of different ages. Tullett (1981) found these changes are because of the number and cross-sectional area of the pores and not due to the thickness of the eggshell. Ipek

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& Sahan (2001) found that eggs were obtained from different broiler flock ages with different egg weight influences the eggshell quality. Additionally affects the egg weight loss, hatchability performance and chick quality (Bamelis et al 2008). Roque & Soares (1994) reported that eggshell water vapor conductance (GH_2O) also affects hatchability in broiler hatching eggs. Peebles et al (2000) reported that when the flock age increase, weight of egg and yolk sac percentage of embryo increase, whereas the thickness of shell decreases. Vieira & Moran (1999) have also reported that yolk sac percentage of embryos from smaller eggs was greater when compared to larger eggs of similar ages.

The aim of the study was to investigate eggshell traits; including eggshell water vapor conductance, eggshell thickness, pore density, egg weight loss as well as surface area and volume in two broiler breeder ages. Furthermore, the phenotypic correlations among the eggshell characteristics were determined.

2. Material and Methods

2.1. Experimental design and parameters

The care and use of animals were in accordance with the laws and regulations of Turkey and approved by the ethics committee of Uludag University (License Number 2018-05/01). A total of 130 hatching eggs were obtained from commercial Ross 308 broiler breeder flock at 28 wk and 40 wk-old age on the same day. The flocks were reared and kept under standard management conditions according to breeder company recommendation (Aviagen 2010). A standard breeder diet was given to both of the flocks (16.0% CP and 2750 kcal of ME kg^{-1}) between 22 and 65 wk of age. The nutrient content of the diet was prepared according to the NRC (1994) specifications. Eggs were stored at 18 °C and 65% relative humidity for 3 days then pre-warmed for 8 hours at room temperature of 22 ^{o}C. Eighty eggs were incubated to investigate eggshell traits and chick quality parameters. The eggs were ranged from 54.41 g to 60.78 g in 28 wk-old flock age group and from 54.71 g to 60.62 g in 40 wk-old flock age group. The eggs were numbered and weighed with ± 0.01 g precision and were monitored one by one during the incubation period. Eggs were incubated at 37.5 °C and 55% RH in an incubator (setter T640 Cimuka Inc) during 18 days of incubation. On the 18th day of incubation, eggs were weighed to determine egg weight loss and were transferred to the hatcher (Hatcher; Cimuka Inc., Ankara, Turkey). Eggs were kept in separate chambers within trays at 37.0 °C and 60% RH.

2.2. Eggshell thickness

On the 22nd day of the incubation period, the hatched chicks were removed from the hatcher and only the eggshells of hatched eggs were individually stored in the plastic bags for the determination of eggshell thickness and large pore count. Unhatched eggs were opened for visual assessment of fertility and embryonic mortalities. Embryonic mortality was separated into three groups: early term (1 to 7 day of incubation), mid-term (8 to 14 day of incubation), and late-term (15 to 21 day of incubation).

Eggshells from hatched chicks were collected, washed and dried for 24 hours at room temperature. The thickness of eggshell was measured from three different regions of eggs (broad end, equator and narrow end) with the help of ball-point caliper having reading 0.01 mm (Peeble & Cristropher 2013). The thickness of eggshell measurements was conducted by excluded the membranes that were adhered to the eggshell.

2.3. Pore density

Same eggshell regions were also used to measure large pore count. These eggshell regions were dipped in 5% NaOH solutions (gr $liter^{-1}$) for 5 to 10 minutes to remove all shell membrane or other adhered material. Furthermore, for the magnification of pores eggshell regions were dipped in concentrated nitric acid for about 15 seconds. By using aqueous Methylene Blue dye (0.5 g, 89% dye in 1 L of 70% ethanol) the surface of the eggshells were tinted following drying (Board & Halls 1973). In this study, for the determination of pore density, only the large pores were counted under the microscope. The counting field was 0.50 cm^2, and representing three regions (broad end, equator and narrow end) of each egg. For the measurement of average values, estimated values were multiplied by 2 that expressed the pore density per centimeter. Calculation of total surface area formula, $A\ (cm^2) = 4.835\ W^{0.662}$, where W= initial egg mass (g). Likewise estimated values of area and shell thickness were used to calculated the volume of the derived egg by multiplying area and shell thickness (volume is equal to A multiplied by L, where A refers to surface area of the eggs in centimeter square and L refers to thickness of shell in centimeters) (Paganelli et al 1974).

2.4. Conductance

A total of 50 freshly laid eggs from each flock were individually weighed, numbered and there initial weight was recorded to determine only the eggshell water vapor conductance (GH_2O). At 25 ºC the eggs were set in desiccators along with the silica gel to make sure there is zero humidity (Visschedijk 1983). Consecutively for four days, eggs were weighed one by one to measure the rate of weight loss. Water vapor shell conductance was determined in terms of egg mass quantity lost per day for 1 Torr pressure difference across the eggshell, according to the method described by Ar et al (1974). The water vapor pressure inside the egg is saturated and in the desiccators, it is zero. Thus, by dividing the daily mass loss by the saturation vapor pressure of 23.922 torr (25 °C), the water vapor conductance (expressed in mg day^{-1} $torr^{-1}$) is obtained. Weight loss was corrected to a barometric pressure of 1 atmosphere as described by Ar et al (1974).

Water vapor conductance of eggshell was calculated by using the following equations:

$$G_{H_2O} = \frac{M_{H_2O}}{\Delta P_{H_2O}} \quad (1)$$

Where; $G_{H_2O} =$ water vapor conductance (mg * day^{-1} $torr^{-1}$); $M_{H_2O} =$ the rate of weight loss (mg * day^{-1}); $\Delta P_{H_2O} =$ water vapor pressure difference.

2.5. Data analysis

Data were analyzed using descriptive Statistics, t-Test: two-sample as summing equal variances using SAS (2008). Pearson correlation was used to determine the correlation of different parameters of eggshell characteristics. Mortality data was analyzed using chi-square test.

3. Results and Discussion

In this study, two early embryonic deaths were determined in 28 wk flock, whereas one early embryonic death and two late embryonic deaths was found in 40 wk-old flock. Two unfertile eggs were determined in both of the flocks. By considering these values percentages of fertility in 28 wk-old flock and 40 wk-old flock was found to be similar (95%). The mortality did not differ between the two groups. Hatchability of fertile eggs was numerically found higher in 28 wk-old flock (94.74%) than in 40 wk-old flock (92.10%) although it was not significantly different between the groups (chi-square= 0.188. P>0.665). Smilarly Iqbal et al (2016) found reduced hatchability and increased embryonic mortality with the advanced age of the breeder.

Water vapor conductance of 28 wk-old flock and 40 wk-old flocks is presented in Table 1. In the present study, eggshell water vapor conductance was affected by flock age, conductance (G) value was 11.00±1.01 mg H_2O day^{-1} $torr^{-1}$ and 11.99±1.28 mg H_2O day^{-1} $torr^{-1}$ in 28 and 40 wk-old flock, respectively ($P<0.05$). In accordance with Morita et al (2009) who found that eggs obtained from older breeders possess higher water vapor conductance. Christensen et al (2005) also found that young flock hens produce thicker eggshells and light chicks due to a less exchange of gases and lower eggshell conductance than older flocks. French & Tullet (1991) reported that GH_20 shows an increment in the first half of the laying cycle, then becomes moderate and constant, and finally increased again at the end of the laying cycle. O'Dea et al (2004) found that eggs from 37 wk-old flock had higher water vapor conductance compared with eggs from 35-43 wk-old flocks. Meir & Ar (2008) also reported with the increase of flock age, water vapor conductance also shows an increment in layer hens. Meanwhile, Araujo et al (2017) had calculated the conductance of eggshells by using shell fragments from hatched eggs and results showed that water vapor conductance increased with the increasing flock age. In contrast, Nangsuay et al (2016) reported that eggs obtained from young and older flocks did not differ in eggshell conductance. Hocking et al (2009) found that increased conductance facilitates oxygen uptake of the embryo during late period of incubation. Bamelis et al (2008) showed that increasing conductance rate could be the reason for high embryonic mortality. In the present study, late embryonic mortality was observed in only 40 wk-old flock at the same time eggshell conductance was also found statistically higher this flock age.

Table 1- Water vapor conductance in 28 and 40 wk-old broiler breeders (25 °C temperature and zero humidity)

Group	*n*	*Fresh egg wt (g)*	*24 hr egg wt (g)*	*48 hr egg wt (g)*	*72 hr egg wt (g)*	*92 hr egg wt (g)*	*WVC mg*day^{-1}torr^{-1}*
28 week	25	58.06±1.71	57.90±1.73	57.75±1.73	57.60±1.74	57.43±1.74	11.00±1.01
40 week	25	58.78±1.77	58.60±1.77	58.44±1.78	58.29±1.78	58.11±1.79	11.99±1.28
Significance		0.150	0.166	0.169	0.173	0.177	0.004

WVC, water vapor conductance

Eggshell characteristics of hatched eggs in 28 wk-old flock and 40 wk-old flocks are presented in Table 2. The mean shell thickness and pore density were affected by flock age (P<0.01). The mean shell thickness and pore density were found higher in 28 wk-old flock than in 40 wk-old flock. Additionally, both of two regions (broad and equator) of shell thickness and pore density were found higher in 28 wk-old flock (P<0.01). Surface area was numerically higher in 40 wk-old flock (71.40 cm^2 ± 1.83) whereas volume was numerically higher in 28 wk flock (25.32 cm^3 ± 1.75). Both of them are important geometrical calculations for the poultry industry and can be used to predict chick weight, egg hatchability, shell quality characteristics, and egg interior parameters (Zhou et al 2009). The association was related to increased egg size and a decreased eggshell surface area to egg volume ratio (Peebles & Brake 1987).

Table 2- Eggshell characteristics of hatched eggs in 28 and 40 wk-old broiler breeders

Parameters	*28 week[1]*	*40 week[2]*	*Significance*
Initial egg weight, g	57.28±1.75	57.97±1.64	0.091
Egg weight loss, %	9.49±1.31	9.76±1.55	0.432
Thickness, mm	0.36±0.02	0.34±0.02	0.000
Broad end, mm	0.37±0.02	0.36±0.02	0.044
Equator, mm	0.37±0.02	0.32±0.03	0.000
Narrow end, mm	0.33±0.02	0.33±0.02	0.383
Pore density, pore/cm^2	43.2±15.11	30.3±11.51	0.000
Broad end, pore/cm^2	52.6±21.90	36.7±20.00	0.002
Equator, pore/cm^2	46.3±19.62	29.8±12.01	0.000
Narrow end, pore/cm^2	29.1±12.51	24.5±12.90	0.129
Surface area, cm^2	69.99±1.20	71.11±1.45	0.001
Volume, cm^3	25.32±1.75	24.12±1.58	0.003

[1], n= 36 eggs; [2], n= 35 eggs

Gualhanone et al (2012) found that with the increase of age in different flocks shell thickness also tends to decreases. Roland et al (1988) reported that with the increment of hen's age, shell thickness decreases because total shell deposition after the first three months of laying period remains fairly constant while eggs continue to increase in size. It causes a thinner shell and a deterioration of shell quality (Hamilton et al 1979). In contrast to this result, in another study, it was reported that shell thickness was not influenced by flock age (Kontecka et al 2012).

Pore size has an important role for the shell conductance and the determination of the respiratory rate of an embryo. It is estimated that there are between 100 and 300 pores per cm^2 of the shell surface (Gilbert 1971). It can be inferred that eggshell conductance does not depend only on shell thickness. The main factor may be the number of pores, present in the eggshell (Araujo et al 2017). In this study flock age significantly affected the number of pores except for the narrow region (P<0.01). The mean of pore density of eggshells was higher in 28 wk-old flock age than in 40 wk-old flock (Table 2). In contrast, Shanawany et al (1984) and Araujo et al (2017) reported that older breeders produce eggs with higher total pore number than young flock ages. These results are in contrast to our findings because only large pores were counted. The mean pore of two regions (broad and equator) were 52.6±21.9, 46.3±19.6 of eggshell were higher in 28 wk-old flock while as in 40 wk-old flock it was found 36.7±20.0, 29.8±12.0 (Table 2). The results were in accordance with Ancel & Girad (1992) who reported that the greater number of the pore in the broad and equator region of eggshell. Meir & Ar (2008) found that porosity is main factor for the determinant of water loss from the egg. Similarly, Deeming et al (2002) reported that egg water loss depends on eggshell porosity, pore number, length and shape of the pore. In this study egg weight loss was not found to be significant in two flock ages. In the present study, it may be concluded that there was not any difference in egg weight loss in between two flock ages, due to lower pore density in the eggshell of 40 wk flock. In contrast, this result Reis et al (1997) showed that eggs from older breeders had higher weight loss than younger breeders. Normally accepted average egg weight loss is 12% while as, Carey et al (1986) reported that individual embryos could tolerate a wide range of condition and they can be successfully hatched. Similarly, in our study despite lower than 12% average egg weight, hatchability was not much affected.

Correlation of eggshell characteristics in 28 and 40 wk-old flocks are shown in Table 3 and 4. A non-significant correlation was found between mean eggshell thickness and mean pore density along with the three regions in 28 wk-old flock age and 40 wk-old flock age. While a positive correlation was found between mean thickness and three regions of eggshell in both flocks (P<0.01). Araujo et al (2017) found a positive correlation only between eggshell porosity and three regions of eggshell in 29, 35, 59 wk flock ages. Peeble & Brake (1987) reported relative porosity of eggs laid during early production may be higher due to their larger surface area to volume ratio. In our study, there are some significant correlations between volume and mean thickness (r= 0.935) and the thickness in three regions of eggshell in 40 wk-old flock. A significant positive correlation was found in between initial weight and volume in 28 wk-old flock. (r= 0.346, P<0.05). There was a positive correlation between mean thickness and volume in 28 wk-old flock age (r= 0.912) and also in 40 wk-old flock age (r= 0.935; P<0.01). A non-significant correlation was found between initial egg weight and eggshell thickness and pore density additionally between mass loss and eggshell thickness and pore density. However, Gualhanone et al (2012) reported that eggshell thickness, egg weight may have a correlation with breeder age.

Table 3- Correlation of eggshell characteristics in 28 wk-old broiler breeder

Parameters		*Thickness*				*Pore mean*				
	Mass loss	*Thickness*	*Broad end*	*Equator*	*Narrow end*	*Pore*	*Broad end*	*Equator*	*Narrow end*	*Volume*
Initial egg wt	-0.230^{NS}	0.062^{NS}	0.051^{NS}	-0.066^{NS}	0.108^{NS}	0.107^{NS}	0.071^{NS}	0.128^{NS}	0.145^{NS}	0.346*
Mass los	-	0.059^{NS}	0.097^{NS}	-0.012^{NS}	0.104^{NS}	-0.007^{NS}	-0.131^{NS}	0.002^{NS}	0.121^{NS}	-0.055^{NS}
Thickness	-	-	0.800**	0.791**	0.871**	-0.065^{NS}	-0.090^{NS}	-0.040^{NS}	-0.274^{NS}	0.912**
Broad end	-	-	-	0.431*	0.624**	-0.109^{NS}	-0.088^{NS}	-0.066^{NS}	-0.287	0.748**
Equator	-	-	-	-	0.550*	0.149^{NS}	0.093^{NS}	0.174^{NS}	-0.211^{NS}	0.683**
Narrow end	-	-	-	-	-	-0.142^{NS}	-0.157^{NS}	-0.174^{NS}	-0.163^{NS}	0.797**
Pore density	-	-	-	-	-	-	0.811**	0.744**	0.503*	-0.172^{NS}
Broad end	-	-	-	-	-	-	-	0.365*	0.318^{NS}	-0.156^{NS}
Equator	-	-	-	-	-	-	-	-	0.309^{NS}	-0.067
Narrow end	-	-	-	-	-	-	-	-	-	-0.306^{NS}

**: $P < 0.01$; *: $P < 0.05$; NS: Not Significant

Table 4- Correlation of eggshell characteristics in 40 wk-old broiler breeder

Parameters	*Thickness*					*Pore Density*				
	Mass loss	*Thickness*	*Broad end*	*Equator*	*Narrow end*	*Pore density*	*Broad end*	*Equator*	*Narrow end*	*Volume*
Initial egg wt	0.008^{NS}	-0.223^{NS}	-0.125^{NS}	-0.108^{NS}	-0.244^{NS}	-0.113^{NS}	-0.084^{NS}	-0.093^{NS}	-0.088^{NS}	0.014^{NS}
Mass loss	-	-0.225^{NS}	-0.348*	-0.082^{NS}	-0.030^{NS}	0.093^{NS}	0.123^{NS}	-0.053^{NS}	0.105^{NS}	-0.301^{NS}
Thickness	-	-	0.729**	0.709**	0.595**	0.091^{NS}	0.075^{NS}	0.156^{NS}	-0.018^{NS}	0.935**
Broad end	-	-	-	0.292^{NS}	0.212^{NS}	-0.039^{NS}	-0.031^{NS}	-0.054^{NS}	-0.007^{NS}	0.720**
Equator	-	-	-	-	-0.071^{NS}	0.122^{NS}	0.179^{NS}	0.168^{NS}	-0.102^{NS}	0.689**
Narrow end	-	-	-	-	-	0.111^{NS}	-0.004^{NS}	0.208^{NS}	0.107^{NS}	0.483*
Pore density	-	-	-	-	-	-	0.805**	0.695**	0.786**	0.086^{NS}
Broad end	-	-	-	-	-	-	-	0.244^{NS}	0.379*	0.101^{NS}
Equator	-	-	-	-	-	-	-	-	0.556*	0.124^{NS}
Narrow end	-	-	-	-	-	-	-	-	-	-0.042^{NS}

**, P<0.01; *, P<0.05; NS, Not Significant

4. Conclusions

Eggshell quality such as conductance, thickness, and pore density are an important characteristic that affects the embryonic gas exchange and development of the embryo. The relationship between these parameters such as, eggshell conductance and flock age should be taken in consideration for debating their impacts on hatching output. Use of desiccators for determining the eggshell conductance before incubation most importantly for different age groups is a beneficial tool for selecting the appropriate scenario of incubation including temperature and relative humidity to achieve the better results of hatching and growth of hatched chicks. It is suggested that the broiler breeder should determine the functional properties of the particular eggs that they are using so that hatchability and hatching quality can be improved by matching incubator conditions to these eggs.

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